

Antibacterial behaviour of Common Winter Season Weeds on Human Pathogens in Doon Valley, Uttarakhand, India

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Abstract: Weeds are plants which over dominate others by abundance, growth, and coverage, thus disturbing of ecosystems. Weeds are adapted well to grow in both unfavourable and favourable environments and cause yield and quality loss, competing with crops for resources. The resistance behaviors against microbial diseases promote us to find the antimicrobial behavior of weeds grown in Dehradun during winter season against common pathogens. Out of the twelve extracts assayed, the extract of *Euphorbia helioscopia* was most active against all the studied bacterial strains. Therefore, Minimum Inhibitory Concentration (MIC) of this extract was determined against the selected bacteria. The MIC for different strains is 62.5mg/ml. Besides having antimicrobial activities, plants also be used as a source of anti diabetic and antioxidant potential due to presence of glycosides, tri terpenoids etc.

Keywords: Antibacterial activity; Weeds; Pathogens, Minimum inhibitory concentration

1. Introduction

Plants, since ancient times have been utilized as a source of nutrition and healthcare products. Plants are a reservoir of diverse kinds of bioactive chemical agents and have often been utilized either in the form of traditional preparations or as pure active principles. It is reasonable to make use of locally available plants, domesticated or wild, that could substitute the synthetic preparations. The healing powers of traditional herbal medications have been validated by various workers. The use of herbs as complementary and alternative medicine has increased dramatically in the last 20–25 years [1]. Moreover, emergence of multiple drug resistant strains of microorganisms due to indiscriminate use of antibiotics has generated a renewed interest in herbal medicines [2].

Weeds, commonly defined as a plants that grows out of place and are competitive, persistent and pernicious[3,4], have been a part of civilization and many ancient documents mention about humans battling weeds in the crop fields. In a broad sense, weeds are plants which over dominate others by abundance, growth and coverage, thus disturbing the balance of ecosystems [4, 5]. Due to their inherent efficiency in nutrient uptake and use, weeds easily invade disturbed lands, adversely affecting ecosystems and also disturb /reduce natural phyto - diversity. Weeds are adapted well to grow in both unfavourable and favourable environments and cause yield and quality loss, while competing with crops for resources [6, 7].

Weeds are also found to be resistance to most of the microbial disease when compared to the crops which shows disease symptoms. This antimicrobial behavior is associated with production of various allelopathic interactions. Different types of allelochemicals (secondary compounds) may act as the eco friendly agrochemicals useful as fungicide, pesticide, and herbicides.

Moreover, modern medicine is becoming more widespread but at a high cost. In rural areas, it is useful in certain cases, like surgery, but traditional medicine still plays an important role and is commonly used as primary form of health care for people especially in critical cases such as, in postpartum women, A number of plant species have been used in traditional medicine to treat inflammations, bronchitis, cold, and infections or as a diuretic, antipyretic, analgesic, emetic etc. Considering the aforesaid, it is assumed that the need of the hour is to search for new antimicrobials [8-12]. Keeping in view above biological activities and resistance behavior against microbial diseases promote us to find the antimicrobial behavior of weeds grown in Dehradun, India during winter season associated with crops like wheat and mustard.

2. Material and Methodology

2.1 Materials

The material for the present study comprised of whole plants of common weeds of Dehradun such as *Anagallis arvensis* L., *Capsella bursa-pastoris* (L.)Medik., *Chenopodium album* L., *Euphorbia helioscopia* L., *Fumaria indica* (Hausskn.) Pugsley, *Gnaphalium canescens*, *Plantago major* L., *Ranunculus sceleratus* L., *Rumex nepalensis* L., *Saussurea heteromalla*, *Silene conoidea* (L.), *Stellaria media*(L.)Vill. that grows in association with winter crops like wheat and mustard in Dehradun, Uttarakhand, India.

2.2 Collection and processing of plant samples

The fresh whole plants were collected from different places of Doon valley, Uttarakhand, India. The plant samples were dried in shade at 25^oC to 35^oC for 15-20 days in the laboratory and then crushed to coarse powder using grinder. The dried plant material was stored in paper bags.

The dried plant materials powder was subjected to diffusion extraction with ethanol solvent. About 50gm accurately weighed dry plant samples powder were extracted with 200ml of respective solvent in a closed flask and allowing it to stand for 48 hours. After the extraction process, the filtrates were separated by filtration and the solvent were evaporated using water bath. The extract was then transferred to previously weigh air tight container, (weighed on an electronic balance), percentage yield of the crude extracts were calculated and stored in refrigerator until they were screened for the antibacterial activity.

2.3 Source of Bacterial Cultures

The antibacterial assay of different extracts of studied plants were performed against four bacterial strains. All the bacterial strains were procured from the Department of Microbiology, S.G.R.R.I.T.S., Dehradun, Uttarakhand. The study was carried out at the Microbiology laboratory in Department of Life Sciences, S.G.R.R.I.T.S., Dehradun, Uttarakhand, India.

2.4. Evaluation of the Antibacterial Potential of Plants Extracts

A total of four bacterial strains i.e. two gram positive viz. *Staphylococcus epidermidis*, *Staphylococcus aureus* and two gram negative bacteria viz. *Salmonella typhimurium*, *Pseudomonas aeruginosa* were taken to evaluate antibacterial potential of the different extracts of weeds. The bacterial cultures were maintained on normal nutrient agar slants that were stored at 4°C.

2.5. Antibacterial Screening

Antibacterial activity was demonstrated using disc diffusion method [5]. A pure colony of each of the test bacterial strains were sub cultured into 5mL of nutrient broth using inoculating loop followed by incubation at 37°C for 3-4 hours. The dried extracts were dissolved in dimethyl sulfoxide (DMSO) and subjected to preliminary antibacterial screening with the concentration of 500mg/ml to know whether they were active against the particular bacteria or not. 0.1ml of inoculum was spread on the solidified Muller Hinton agar surface. For screening of active extracts, sterile 6mm diameter whatman No. 41 filter paper discs were dipped in the plant extracts and then placed carefully onto the Muller Hinton agar media against the studied bacterial strains. The results were recorded by measuring the zones of growth inhibition surrounding the disc. Clear inhibition zones around the discs indicate antibacterial activity.

Further, Minimum Inhibitory Concentration (MIC) value of only active extracts were determined against those bacterial strains which showed high sensitivity during the preliminary antibacterial testing. It was performed by serial dilution of the active concentrated extract in pure DMSO to achieve a decreasing concentration range of 500mg/ml to 31.25mg/ml. The sensitivities of all the bacterial strains were also observed against the standard drug such as streptomycin and against DMSO to set them as a positive and a negative control, respectively.

3. Result and Discussion

Weeds are commonly grown among the cultivated plants, may be used for food or medicinal purposes viz., *Portulaca oleracea* L. and *Mentha spicata* L. are examples among a great many others, as they not create any side effect to the human's body while chemical based antibiotics does so. In the same direction studies was conducted by various scientist such as Bhuvaneswari *et al.* (2011), Bhattarai & Shreshta, (2009), Kannan and Kulandaivelu (2007), Kumar Samy *et al.*, (2002), Parekh *et al.*, (2005), Singh *et al.*, (2013), Uday Prakash, (2011) [8,9,10,11,12,13,14] on comparison of antimicrobial activities of weeds such as *Withania*, *Eupatorium* etc. and have claimed that the antimicrobial activity might have been observed due to the presence of strong inhibitory allelochemicals present in these invasive weeds. These compounds exhibit interesting biological properties, such as phytotoxic, antimicrobial, antifungal, and insecticidal properties. These chemicals, in addition to a wide variety of related compounds, are involved in their metabolism, detoxification mechanisms, and degradation on crop soils and other systems [15].

The present study was an effort to investigate and validate the important above mentioned potentials of the weeds in regards to determine the antibacterial activity of common winter season weeds of Doon Valley, Uttarakhand.

3.1 Nature and yield of crude extracts

Different quantities of the powdered plants material were subjected to solvent extraction. The extracts were concentrated on water bath and they were finally reduced to dryness to get dry extracts. The maximum percentage of crude extract yield is obtained in *Stellaria media* (10.70%) followed by *Plantago major* (7.734%) (Table1).

Table 1: Yield of crude extracts of weeds from ethanol solvent

Weed plants	Weight of extract (gm)	Percentage Yield (%)
<i>Anagallis arvensis</i> L.	2.45	5.34
<i>Capsella bursa pestoris</i> (L.) Medik.	1.17	4.87
<i>Chenopodium album</i> L.	1.82	3.63
<i>Euphorbia helioscopia</i> L.	3.18	6.35
<i>Fumaria indica</i> (Hauskn.)Pugsley	2.26	6.84
<i>Gnaphalium canescens</i>	2.24	4.48
<i>Plantago major</i> L.	1.27	7.74
<i>Ranunculus sceleratus</i> L.	1.02	2.04
<i>Rumex nepalensis</i> L.	1.14	4.90
<i>Saussurea heteromalla</i>	2.17	4.33
<i>Silene conoidea</i> (L.)	0.69	3.01
<i>Stellaria media</i> (L.) Vill.	2.03	10.7

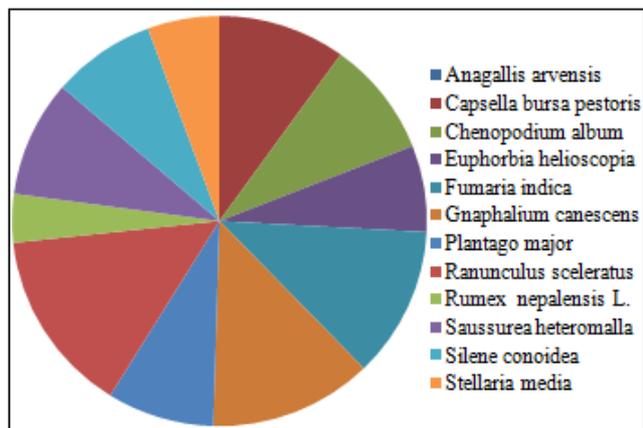


Figure 1: Comparative analysis of percentage yield of crude extract of weeds of winter season

3.2 Antibacterial Assay

All the twelve extracts of plants showed antibacterial activity against all the studied bacterial strains that are pathogenic to human beings causing several diseases such as pneumonia, food poisoning, urinary tract infections, meningitis, fever etc. Among the extracts assayed, *Euphorbia helioscopia* were found to be most active that exhibit antibacterial activity against all bacterial strains. In contrast to this, all plant extracts exhibited highest activity against *Pseudomonas aeruginosa* followed by

Staphylococcus aureus among all the studied bacterial strains (Table 2). While Coban and Biyik (2010) [16] has reported antibacterial activities of *Ranunculus* against various bacterial strains and found higher antibacterial activity as comparison to others.

Table 2: Zone of inhibition (mm) of plant extracts against the bacterial strains

Weed Plants	Zone of inhibition (in mm) against Bacterial Strains			
	SE	SA	ST	PA
<i>Anagallis arvensis</i> L.	1	2	1	8
<i>Capsella bursa pestoris</i> (L.) Medik.	8	2	1	7
<i>Chenopodium album</i> L.	1	7	1	9
<i>Euphorbia helioscopia</i> L.	9	7	8	15
<i>Fumaria indica</i> (Hauskn.)Pugsley	1	2	1	8
<i>Gnaphalium canescens</i>	3	1	-	7
<i>Plantago major</i> L.	1	1	1	9
<i>Ranunculus sceleratus</i> L.	1	9	1	10
<i>Rumex nepalensis</i> L.	3	2	1	8
<i>Saussurea heteromalla</i>	9	7	1	-
<i>Silene conoidea</i> (L.)	1	3	1	10
<i>Stellaria media</i> (L.) Vill.	2	9	8	11

Disc Diameter: 6mm (included)

Note: *SE: *Staphylococcus epidermidis*; SA: *Staphylococcus aureus*; ST: *Salmonella typhimurium*; PA: *Pseudomonas aeruginosa*

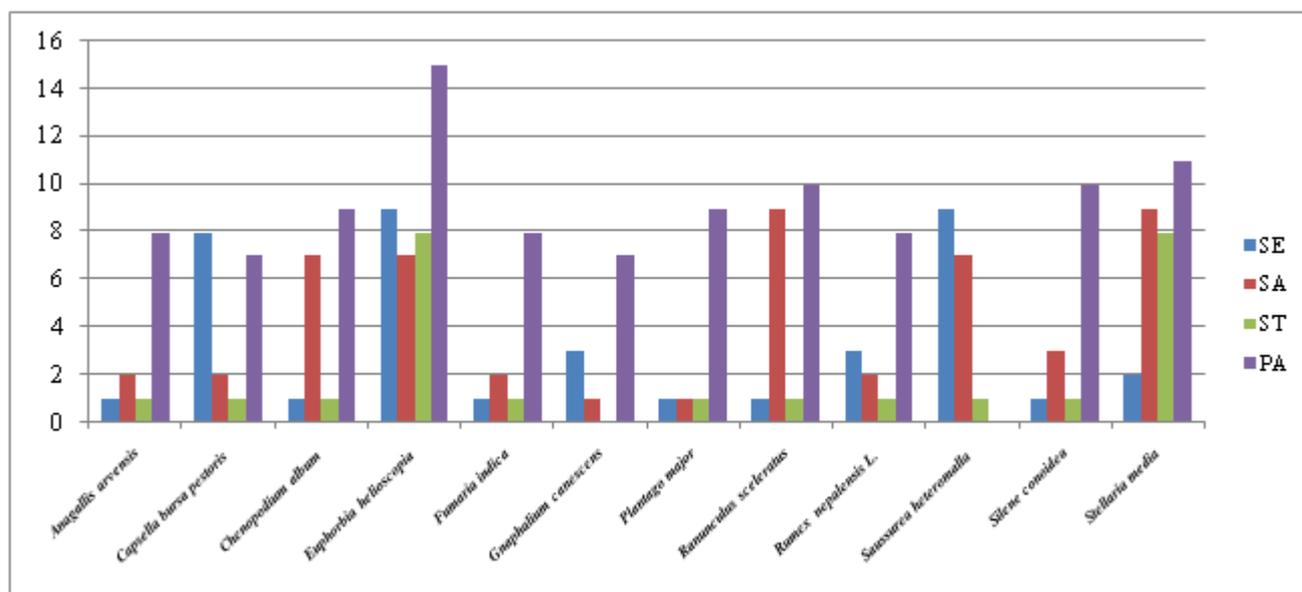


Figure 2: Comparative analysis of zone of inhibition of plant extracts against four bacterial strains

Out of the twelve extracts assayed, the extract of *Euphorbia helioscopia* was most active against the studied one Gram negative and two Gram positive bacterial strains. The maximum zone of inhibition obtained during the preliminary screening for extract was 15mm against *Pseudomonas aeruginosa*, 7mm against *Staphylococcus aureus*, 8mm against *Salmonella typhimurium* and 9mm against *Staphylococcus epidermidis*. Therefore, Minimum Inhibitory Concentration (MIC) of this extract was determined against the selected bacteria (Table 3).

3.3 Minimum inhibitory concentration (MIC) analysis

Minimum inhibitory concentration is important in diagnostic laboratories to confirm resistance of microorganisms to an antimicrobial agent and also to monitor the activity of new antimicrobial agents. A lower MIC is an indication of a better antimicrobial agent [17]. An MIC is generally regarded as the most basic laboratory measurement of the activity of an antimicrobial agent against an organism [18]. MIC analysis was performed by serial dilution of the concentrated ethanol extract in pure DMSO to achieve a decreasing concentration range of 500mg/ml to 62.5mg/ml.

On performing MIC analysis for the *Euphorbia helioscopia* extract, the results revealed that all the selected bacterial strains i.e. *Pseudomonas aeruginosa* were sensitive against the 62.5mg/ml concentration of the extract, thereby exhibiting 62.5mg/ml as their MIC value except *Staphylococcus epidermidis*, and *Staphylococcus aureus*, *Salmonella typhimurium* that were sensitive against the 250mg/ml concentration of the extract whose MIC was 250mg/ml (table-3). In order to check the susceptibility of bacterial strains ready to use antibiotic impregnated disc i.e. Streptomycin was used as a positive control. All of them showed clear zones of inhibition around the disc interpreting their high sensitivity towards antibiotic. In contrast to this, DMSO (99% pure) was used as a negative control against which none of the bacterial strains showed zone of inhibition revealing resistance of cultures towards DMSO (table-4).

Table 3: Minimum Inhibitory Concentration (MIC) of *Euphorbia helioscopia* extract

Bacterial Strains	Zone of Inhibition in mm			
	500 mg/ml	250 mg/ml	125 mg/ml	62.5 mg/ml
<i>Staphylococcus epidermidis</i>	9	7	-	-
<i>Staphylococcus aureus</i>	7	4	-	-
<i>Salmonella typhimurium</i>	8	5	-	-
<i>Pseudomonas aeruginosa</i>	15	13	10	7

Table 4: Zone of inhibition (mm) of positive and negative control against the bacterial strains

Bacterial Strains	Negative Control: Streptomycin	Positive control: DMSO
<i>Staphylococcus epidermidis</i> MTCC-5615	20mm	-
<i>Staphylococcus aureus</i> MTCC-737	24mm	-
<i>Salmonella typhimurium</i> MTCC-1255	26mm	-
<i>Pseudomonas aeruginosa</i> ATCC-424	25mm	-

Disc Diameter: 6mm for positive and negative control (included).

4. Conclusion

The present study was carried out on the antibacterial activity of common winter season weeds in Doon valley against the pathogenic bacterial strains. Out of the twelve extracts assayed, the extract of *Euphorbia helioscopia* was most active against all the studied Gram negative and Gram positive bacterial strains. Therefore, Minimum Inhibitory Concentration (MIC) of this extract was determined against the selected bacteria. The MIC value for *Pseudomonas aeruginosa* is 62.5mg/ml whereas for *Staphylococcus epidermidis*, and *Staphylococcus aureus*, *Salmonella typhimurium* 250mg/ml. In future the plant extract of weeds could be exploited to control various bacterial infections as plant extracts revealed the presence of various phyto-constituents such as glycosides, tri terpenoids, flavanoids etc.

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