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COMPARATIVE ANTIBACTERIAL POTENTIAL OF ETHANOLIC EXTRACTS OF MEDICINAL PLANTS FROM HIMACHAL PRADESH, INDIA

Shikha Rangra Chandel[1*], Shikha Guleria[2], Kumari Shalini[1], Kiran Kumari [1], Divya Bharti [2], Deeksha Kumari[1], Manisha Kumari[1], Anita Kumari[1] and Rajan Rolta[2]

[1*]Department of Microbiology, Career point university, Hamirpur (H.P.), India.
[2]Faculty of Applied sciences and Biotechnology, Shoolini University of Biotechnology and Management Sciences, Bajhol, PO Sultanpur, District Solan-173229 (Himachal Pradesh), India.

Abstract

Medicinal plants are also known as medicinal herbs and used in traditional medicines practices. Medicinal plants are used in non-industrialized societies, mainly because they are readily available and cheaper as compare to modern medicines. A vast number of medicinal plants have been recognized as valuable resources of natural antimicrobial compounds as an alternative that can potentially be effective in the treatment of these problematic bacterial infections. The most common bacterial agents including *B. subtilis, E. coli* and *P. aeruginosa* are responsible for several human infections.

Around 90% of the people of Himachal Pradesh live in villages with diverse culture, communities with specific traditional knowledge of the each region. Medicinal plants of Himachal Pradesh in order to treat various bacterial diseases, eradicating side effects of allopathic medicine through proper identification and characterization of medicinal plants. Plants are rich in a wide variety of secondary metabolites such as tannins, alkaloids, phenolic compounds, and flavonoids, which have been found *in vitro* to have antimicrobial properties. The present study was aim to evaluate antibacterial potential in the ethanolic extracts from leaves of *Biden pilosa, Bryophyllum pinnatatum* and *Lantana camara.* The antibacterial activity of selected plants was checked by using agar well diffusion method. The ethanolic extract of leaves of *B. pilosa, B. pinnatatum* and *L. camara* exhibited more antibacterial activity against gram-ve bacteria as compare to gram +ve bacteria. The maximum zone of inhibition was observed in *B. pinnatatum* against *P. aeruginosa*. The minimum Inhibitory concentration for leaves *of L. camara* were 2.5 mg, 2.5 mg and 0.156 mg against *B. subtilis, E. coli* and *P. aeruginosa* respectively. The present study provides the evidence for antibacterial potential of medicinal plants. Moreover, this type of study provides the medicines at affordable cost.

Key words : Medicinal plants, Antibacterial, *Bacillus subtilis, Escherichia coli* and *Pseudomonas aeruginosa.*

Introduction

Medicinal plants are the nature's gift to human being to make disease free healthy life. Herbal medicine is still the mainstay of about 75-80% of the whole population, mainly in developing countries, for primary health care because, better compatibility with the human body and fewer side effects (Barmet, 1992). Over 50% of all modern drugs are of natural product origin and they play an important role in drug development programs of the pharmaceutical industry (Baker *et al.,* 1995). A vast number of medicinal plants have been recognized as valuable resources of natural antimicrobial compounds as an alternative that can potentially be effective in the treatment of these problematic bacterial infections (Iwu *et al.*, 1999).

Antimicrobial agents are essentially important in reducing the global burden of infectious diseases (Bhatia and Narain, 2010). However, emergence and dissemination of multidrug resistant (MDR) strain in pathogenic bacteria have become a significant public health threat as there are fewer, or even sometimes no,

Author for correspondence : E-mail: shikha777rangra@gmail.com

effective antimicrobial agents available for the infection caused by pathogenic bacteria (Boucher *et al.,* 2009; Giamarellou, 2010).

According to the World Health Organization (WHO), medicinal plants would be the best source to obtain a variety of drugs (World Health Organization, World Health Organization, WHO Traditional Medicine Strategy, Geneva, 2002 (WHO 2002). Plants are prospective source of antimicrobial agents in different countries (Alviano and Alviano, 2009) Recent emergence of antibiotic resistance and related toxicity issues limit the use of antimicrobial agents (Eggleston *et al.,* 2010) and is prompting a revival in research of the antimicrobial role of plants against resistant strains due to comparable safety and efficacy (Alviano and Alviano, 2009)

Materials and Methods

The present study was focused on the antibacterial potential of medicinal plants i.e. *Biden pilosa, Bryophyllum Pinnatum* and *Lantana camara* collected from Hamirpur district of Himachal Pradesh. Flow diagram is shown in fig. 1.

Collection and Sterilization of Plant Materials

The leaves of *B. pilosa, B. Pinnatum and L. camara* were collected from different location of Hamirpur, Himachal Pradesh. All the three location were randomly picked for sampling. The leaves of *B. pilosa, B. Pinnatum and* L. *camara* were collected in month of February. Approximately 500 gm each of leaves were harvested from each herb and were properly labelled. The collected plant materials were systematically washed under running tap water to remove dust particle, soil and their unwanted substances. After that leaves samples were sterilized with 70% ethanol and then drying in hot air oven at 37 ºC for a week. Then the samples were powdered with the help of electric grinder and stored in air tight bottles in the dark till use.

Preparation of leaves extract of medicinal plants of Himachal Pradesh

The ethanolic extract of each leaves samples (30 gm) *B. pilosa, B. Pinnatum and L. camara* were prepared by using the maceration process (Eggleston *et al.,* 2010). Then each leaves sample were dissolved in absolute ethanol (100 ml) in a conical flask, plugged with cotton wool and kept on a rotary shaker at 120 rpm for a week to ensure complete extraction. The extracts were

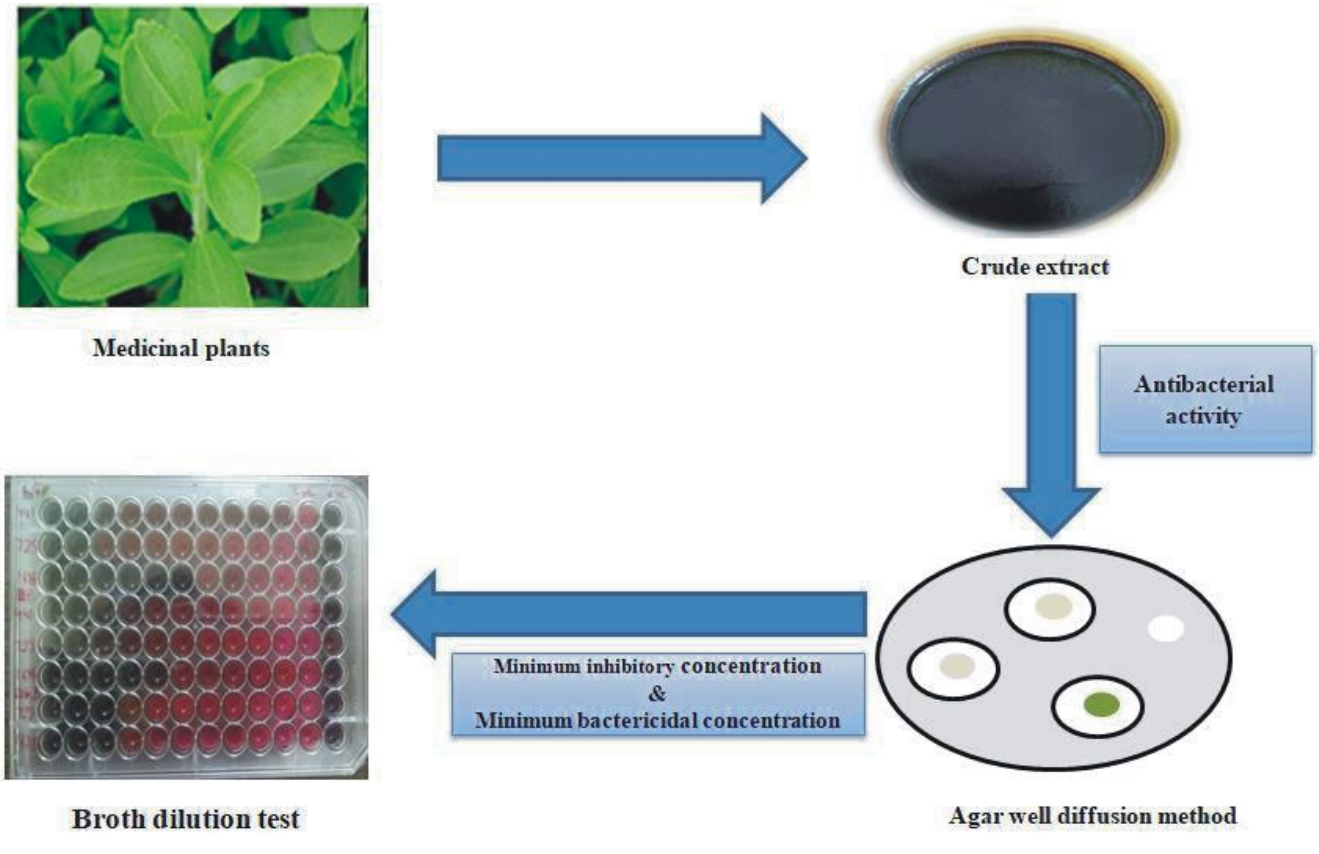

Fig. 1: Medicinal plants showing the antimicrobial activity.

filtered through the whatmann No.1 filter paper. The filtrate was collected and allowed to evaporate at 40 ºC, to yield thick and viscous residues as crude extracts. The dried crude extracts were stored at 4 ºC in air tight bottles till further use.

Phytochemical Screening of ethanolic extract of medicinal plants of Himachal Pradesh

Detection of carbohydrates

A) Molisch's test: 1 ml of Molisch reagent was added to 1 ml of extract followed by mixing and incubating in a boiling water bath for 5 min. A red violet ring is formed between two layers indicates the presence of carbohydrates.

B) Fehling test: To 1 ml of the filtrate, 1 ml of freshly prepared Fehling solution and heated in a water bath. Brick red precipitates indicated the presence of carbohydrates (Ramakrishnan, 1994)

Detection of Protein

A) Millon test: A few drops of concentrated Millons reagent were added by the side of the test tube containing 1 ml of extract and then heated in water bath for 10 min. Formation of Red color indicated the presence of proteins (Rasch and Swift. 1960).

B) Ninhydrin test: To 1 ml of extract, 1 ml of 2% ninhydrin reagent was added and boiled for few min. Appearance of blue color indicated the presence of amino acids (Chandel *et al.,* 2016).

Detection of phenolics and Tannis

A) Ferric chloride test: 30 µl of ferric chloride solution was added to the 1ml of extract. Appearance of blue color indicates the presence of hydrolysable tannins, while the green color indicates the presence of condensed tannins.

B) Gelatine test: 50 µl of 1% gelatin in 10% NaCl was added to 1 ml of extract. Formation of white precipitates indicated the presence of tannins (Guleria *et al.,* 2016).

Detection of Flavonoids

A) FeCl3 test: 1 ml extract was added to 10% $FeCl_3$ (500 µl) and mixture was shaken. A thick brownish precipitate indicates the presence of flavonoids (Mace 1963).

Detection of Alkaloids and Glycosides

For the detection of alkaloids and glycosides, 50 mg of extract was dissolved in 5 ml of dilute HCl and then filtered. The filtrate was used for the detection of alkaloids and glycosides.

A) Dragendorff's Test: To 1 ml of the filtrate, 500 µl of Dragendorff's reagent was added along the sides of the test tube. Formation of orange or orange reddish precipitates indicated the presence of alkaloids (Waldi, 1965).

B) Hager's Test: To 1 ml of the filtrate, 500 µl of Hager's reagent was added. Formation of yellow precipitates indicated the presence of alkaloids (Wagner and Bladt 1996)

Detection of Saponins

A) Foam test: 200 µl extract was mixed with 5 ml distilled water. It was shaken vigorously for 5 min. Persistence of foam indicates the presence of

Detection of steroids

A) Liebermann-Burchard reaction: Liebermann-Burchard reaction was performed for checking the presence of steroids. To 1 ml of extract, 2 ml of acetic anhydride was added and heated to boil, cooled and then 1 ml of concentrated sulfuric acid was added along the sides of the test tube. A blue green ring indicated the presence of steroids (Chandel *et al.,* 2016).

B) Salkowski test: In this test, 0.2 ml of extract was added to 2 ml of chloroform. After 2 min, 20 µl of H_2SO_4 was added along the sides of the test tube (Chandel *et al.,* 2016).

Antibacterial activity of ethanolic extract of medicinal plants of Himachal Pradesh

Qualitative analyses of antibacterial activity by agar well diffusion

Antibacterial activity was tested by using agar well diffusion method (Chandel *et al.,* 2019). About 25 ml of Nutrient agar medium was poured in sterile 100 mm petri-dish, and allowed to solidify. Then, fungal culture of optical density of 0.12~0.15 at 530 nm equivalent to 0.5 McFarland standard was uniformly spread on the surface of the nutrient agar medium using sterile cotton swabs and allowed to absorb. The wells were punched with the cork borer (6 mm) in the agar and 50 µg extract was loaded in the wells and allowed to diffuse. Assay plates were incubated of 18-24 h at 37 ºC and the zone of inhibition was measured using Hi Antibiotic Zone scale-C (Himedia Biosciences, Mumbai (India). Amoxicillin (10 mg) was used as positive control and ethanol (solvent was used as negative control in the antibacterial assay. The tests were performed in triplicate and results were recorded as mean ± SD.

Quantitative analyses of antimicrobial activity by broth micro dilution method

Minimum inhibitory concentration (MIC) was done by broth micro dilution method (Chandel *et al.,* 2016;

Rolta *et al.*, 2018 a,b; Rolta *et al.,* 2020). Minimum inhibitory concentration of plant extracts was determined by the color change of resazurin dye from purple to pink. Amoxicillin was used as positive control and DMSO used as negative control.

All the experiments were performed in triplicate and results were analyzed by mean and standard deviations.

Results

Extract Preparation and Extractive Yield

The color of leaves extract was observed to be dark green in all the selected samples, after completed drying at 37 ºC. Then, there extracts were further subjected to check antibacterial potential. In comparative study the maximum yield of 8.06 ± 0.95% was observed in leaves sample of *B. pinnatum*, while minimum yield of 4.9 ± 8.87 % was found in leaves sample of *L. camara* (Fig. 2).

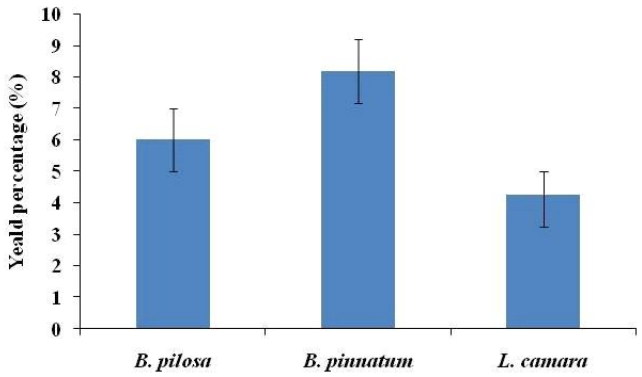

Fig. 2: Extract yield (%) of leaves of *Biden pilosa, Bryophyllum pinnatum and Lantana camara.*

Table 1: Phytochemical screening of ethanolic extract of leaves of *Biden pilosa, Bryophyllum pinnatum and Lantana camara.*

S.No.	Phytochemicals	Test performed	*Biden pilosa*	*Bryophyllum pinnatum*	*Lantana camara*
1.	Carbohydrates	Molisch's test	+	+	+
		Fehling test	+	+	+
2.	Proteins	Million test	+	+	+
3.	Phenols	Ninhydrin test	+	-	+
		Ferric chloride test	+	+	+
		Gelatin test	+	+	+
4.	Flavanoids	Shinoda test	+	+	+
		Alkaline reagent test	+	+	+
5.	Alkaloids	Hager 's test	+	+	+
		Dragendroff test	+	+	+
6.	Tannins	Ferric chloride test	+	+	+
		Lead acetate test	+	+	+
7.	Saponins	Foam test	+	-	+

Phytochemical Screening

The qualitative phytochemical screening showed that thephenols, carbohydrates, flavonoids, alkaloids, saponins, glycosides, tannins and proteins were present in the ethanolic extract of leaves of *Biden pilosa, Bryophyllum pinnatum and Lantana camara* (Table 1).

Antibacterial Potential

The maximum zone of inhibition is shown by the *P. aeuroginosa* as compare to other bacterial strains (*B. subtilis and E. coli*) against *B. pilosa, B. pinnatum and L. camara.* The maximum zone of inhibition is shown by the *P. aeuroginosa* against *B. pinnatum* (Fig. 3). In general, we observed that the zone of inhibition increased with the increase concentration of ethanolic extract of *B. pilosa, B. pinnatum and L. camara.*

Minimum inhibitory concentration

We observe that, the minimum inhibitory concentration of *lantana camara* against the bacteria *(P. aeruginosa*) is 0.156 mg and maximum inhibitory concentration is 2.5 mg of the plant *lantana camara* against the bacteria (*B. subtilis* and *E. coli*) as shown in table 2.

Minimum Bactericidal Concentrations of different medicinal plant of

In case of L. *camara* and *B. pilosa* all the concentration (5 mg, 2.5 mg, 1.25 mg and 0.625mg) are bactericidal whereas, in *B. pinnatum* only one concentration (5 mg) is bactericidal and other concentration (2.5 mg, 1.25 mg and 0.625 mg) are bacteriostatic against the *P. aeuroginosa*. In case of lantana camara the two concentrations (5 mg, 2.5mg) are bactericidal and other two concentrations (1.25mg, 0.625 mg) are bacteriostatic against the *E. coli* bacteria and in case of *Biden pilosa* only one concentration is bactericidal and other three concentration (2.5 mg 1.25 mg and 0.625mg) are bacteriostatic against the *E. coli* bacteria. In case of *Lantana camara* and *Biden pilosa* only one concentration (5 mg) is bactericidal and other three concentrations (2.5 mg, 1.25 mg and, 0.625 mg) are bacteriostatic against the *B. subtilis* bacteria and in case of *B. pinnatum* two concentrations (1.25 mg, 0.625 mg) are against the bacteria *B. subtilis* (Fig. 4).

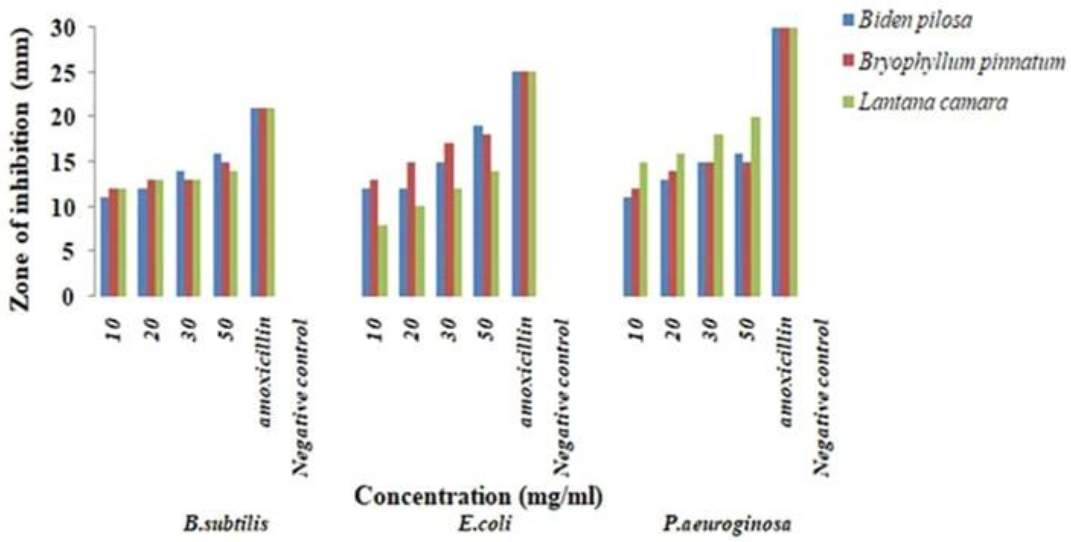

Fig. 3: Graphical Representation of comparative antibacterial activity of *Biden pilosa, Bryophyllum pinnatum and Lantana camara.*

Table 2: Showing the minimum inhibitory concentration of ethanolic extracts of different plants.

Medicinal Plants	Minimum inhibitory concentration (mg\ml)		
	B. subtilis **(MTCC-441)**	***E. coli*** **(MTCC-739)**	***P. aeruginosa*** **(MTCC-1688)**
B. pilosa	1.2	1.25	0.3125
B. pinnatum	1.25	1.25	1.25
L. camara	2.5	2.5	0.156

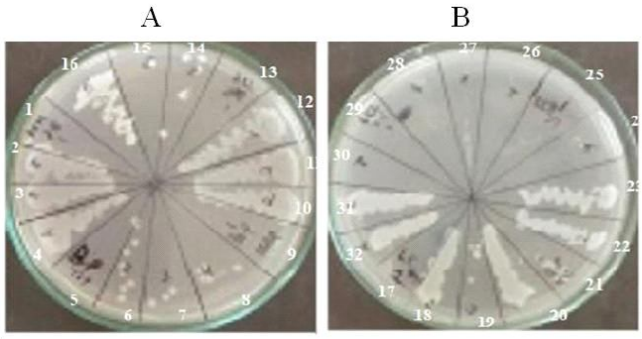

Fig. 4: Minimum bactericidal concentration (a) *B. pilosa & B. pinnatum against the* bacterial strains MTCC-441(1-4), MTCC-739(5-8), MTCC- 1688 (9-12) and MTCC-441(13-16) *respectively*. (b) *B. pinnatum & L. camara* against the bacterial strains MTCC-739 (17-20), MTCC-1688 (21-24), MTCC-441(25-28) & MTCC-739 (29-32) respectively.

Discussion

The findings of the present study showed the differences between the antibacterial activity of ethanolic extract of selected medicinal plants *i.e. B. pilosa*, *B. pinnatum* and L. *camara.* This suggests that these plants have different antibacterial activity against different bacterial strains *i.e. B. subtilis* (MTCC-441), *P. aeuroginosa* (MTCC-1688) and *E. coli* (MTCC-739). *Bidens pilosa* showed maximum inhibition against *P. aeuroginosa* whereas in previous study, the methanolic extract of *Bidens pilosa* shows minimum inhibition to *B. subtilis (*Dagawal and Ghorpade 2011) and the leaf extract of *B. pilosa* showed very good activity against *Salmonella typhi.* (Lawal *et al.,* 2015).

In *Bryophyllum pinnatum*, we found higher antibacterial activity against *E-coli* while in previous study, the methanolic extract of *B. pinnatum* showed inhibition against gram positive bacteria *i.e. Staphylococcus aureus* (Akinsulire *et al.,* 2007). In *Lantana camara*, we observed that maximum inhibition against gram negative bacteria similarly, in contrast to previous study; the *Lantana camara* leaf and flower extract showed antibacterial property against gram negative bacteria i.e. *P. aeuroginosa* and *B. subtilis* (Ganjewala *et al.*, 2009). The ethanolic extract yield of *Bidens pilosa* was 1.76 g whereas in previous study, the methanolic extract of *B. pilosa* was 2.86 g (Lawal *et al.,* 2015).. The ethanolic extract yield of *B. pinnatum* was 1.47 g and 8.03%. As we observed the results of MIC (Minimum Inhibitory Concentration, the *B. pilosa* shows minimum inhibitory concentration *i.e.* 0.3125 mg/ml in case of *P. aeuroginosa*. In contrast, *Lantana montevidensis* leaves extract exhibited better result against *P. aeuroginosa* (MIC 8 µg/ml) (Lawal *et al.,* 2015). In present study, the *Lantana camara* and *Bidens pilosa* have showed Minimum bactericidal concentration whereas in previous study the MBC of leaf extract of *Bidens pilosa* was 1.3 > 10 mg/ml (Lawal *et al.,* 2015).

Conclusion

The extracts showed varying degrees of antibacterial activity on the microorganisms tested. Three different plants (*Bidens pilosa, Bryophyllum pinnatum* and *Lantana camara*) exhibited the difference in the antibacterial activity. These plants showed highest antibacterial activity against *P.aeuroginosa.*This is in vitro study which is demonstrated that traditional medicine can be effective as modern medicine can kill the pathogenic bacteria. We can diagnose the infectious diseases with less or no side effects.

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Conflict of Interest

The authors declare no conflict of interest.

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