Research Article

In Vitro Antibacterial Activity of Medicinal Plant Extracts

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Abstract | Antibacterial activities of four medicinal plants, *Silybum marianum, Berberus lycium, Peganum harmala* and *Curcuma longa* and standard antibiotic tetracycline was carried out in the biosafety level 3 Microbiology Lab, Faculty of Life Sciences, University of Bradford, Bradford, UK. Two different concentrations (low, 5 mg/ml and high extracts, 300 mg/ml) of these plants were evaluate against two different bacterial strains i.e. *Bacillus subtilis and E. coli* using disc diffusion method to uncover antibacterial potential of the plants extracts. The antibacterial investigation was performed in three replicates using disc diffusion technique using Nutrient Agar Media for the growth of *B. subtilis and E. coli* bacteria. The ANOVA for inhibitions zones indicates the effect of plants, bacteria strains, and concentration on growth of bacteria in the bacteria growth culture medium and their interactions. The plants crude extracts were having significant effects on the bacterial growth in bacteria growth medium while bacterial strains and concentrations have also significant affected the bacteria growth and zone of inhibitions by different concentration. However, the various concentration and bacterial strains were having significant effect on bacteria growth at 0.05 level of Probability. The interaction effect of plant bacteria, plant concentration was non-significant and concentration bacteria interactions have significant effect on growth inhibition of Bacteria. However, the three ways interaction between plants, bacteria and concentration was non-significantly affected the bacteria growth in the culture. The mean table indicate all the plants have showed antibacterial activities. The maximum activity was reported in *C. longa* which seem to be most active and lowest value was recorded in *S. marianum*. The maximum inhibition zone has been reported as 20 mm for the *C. longa* extracts and minimum has been observed for *S. marianum* as 13 mm. In case of concentration the maximum inhibition zone 20.58 mm was recorded with 300 mg (high concentration) while 14.6 mm was recorded for 5 mg (low concentration). In case of bacterial strains, the *E. coli* gram positive bacteria was significantly more visible in growth by developing inhibition zone of 20.5 mm while minimum was recorded in *Bacillus* gram negative bacteria that is 14.7 mm. The result indicates that different plant extracts, different concentrations and different bacterial strains may affect bacterial growth inhibition differently. Therefore, further investigation into determining minimum effective concentration, plants specific activity and search for active plant metabolite would explore effective natural therapy or antibiotic for multidrug resistance pathogenic plants and human bacterias.

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Keywords | Antibacterial activity, Bacterial bioassay, Disc diffusion method, Medicinal plant extracts, P. harmala, C. longa

Introduction

There are rich customs of the use of herbal therapy for the treatment of various diseases in the culture of various communities in Pakistan. The knowledge is more with in rural and remote areas with elderly community's member as compare to urban and young population of the communities. There is also an increase in revival of the traditional trend in the use of herbal therapy mainly because of

the low cost and low side effects. The herbal therapy limits the infections by exposing the infections to number of compounds in addition to limiting the side effects of conventional antibiotics. In recent day the issue of resistance to many pathogenic bacteria and to various antibiotic drugs, much concentration has been given to the development and isolation of new and novel active compounds from herbal products. Globally folk used of plants for the treatment of various ailments has been reported for the treatment of viral, bacterial and fungal diseases (Frei *et al*., 1998; Samy and Ignacimuthu, 2000; Kayani *et al*., 2015; Al-Asmari *et al*., 2014; Aziz *et al*., 2017; Ali *et al*., 2017). Further investigations on the traditional reported uses plants are important to validate the information scientifically. So, it is important to carry out biological screening and phytochemical studies of the plants that are in traditional uses for centuries. Most the herbal products currently in used are in raw form or unprocessed form with low concern for the cell toxicity and quality parameter. Recognizing the importance of the herbal treatment in developing countries, World Health Organization has established unit for the promotion, standardization and conservation of traditional medicine. The World Health Organization has also published number of manuscript on traditional herbal medicine

It is believe and in fact that most of the current drugs have origin from the plant base source. The current experiment is an exploration of the plants gather in the ethno-medicinal survey from the various agro-ecological zones and to validate its effectiveness against bacteria. Hence the present experiment was under taken with the following objective:

- To evaluate the antibacterial potential of the selected medicinal plant extracts against bacteria

Materials and Methods

The plants for the experiment were collected from the ethno-medicinal survey of the various agro-ecological zones. The information about it use and it preparation was obtained from herbalist. The plants are reported for it folk and traditional use for the hepatitis and jaundice first time from these regions. The identity of the collected plant was confirmed in the National Herbarium, National Agricultural Research Centre, Islamabad, University of Agriculture Peshawar and Medicinal Plant Herbarium, Pakistan Forest Institute, Peshawar. The specimen of the plants has been deposited in the herbarium of Pakistan Forest Institute, Peshawar.

Plant extract preparation

The plants were chopped to small piece and air dried in shade at room temperature 25 ±3 ^{0}C. Using electrical grinder, the plants were powdered to use for further extraction. 300 mg of dried powder of each plant was taken in 15 ml sterile tube (Tube 15ml, 120x17mm, PP, Sarstedt Germany) dissolved in 1500 µl methanol (99.99% pure), mix thoroughly and kept for 5 minutes. The sample was sonicated for 5 minutes using Fisher brand Ultrasonic FB1103. After sonication the sample was shake occasionally for 20 minutes by vertixing using IKA-VIBRAX-VXR Electronic, Made in Germany. After sonication and vertixing the samples were centrifuge for 10 minutes at 6000 rpm using Hettich Zentirifugen EBA 21 centrifuge, Made in Germany. After centrifuge the supernatant was collected in 15 ml glass vial for further analysis Figure 1.

Antibacterial bioactivity assay

The antibacterial activity of crude methanolic extracts of medicinal plants was carried out using disc diffusion methods this method most widely reported in literature for bacterial bioassay (Essawi and Srour, 2000; Ullah *et al*., 2015). The antibacterial bioactivity assay was involving the screening of *Silybum marianum, Berberus lycium, Peganum harmala* and *Curcuma longa* plants extracts against gram positive and negative bacterial strains. Antibacterial bacterial potential of these plants extracts were evaluated in the biosafety level 3 Microbiology lab Faculty of Life Sciences, University of Bradford, West Yorkshire Bradford UK. The antibacterial investigation was performed in three replicates using disc diffusion technique using Nutrient Agar Media for the growth of *Bacillus subtilis and E. coli bacteria*. The antibacterial assay was conducted to uncover and validate the potential of these medicinal plants extracts to inhibit the growth of bacteria in in vitro culture.

Preparation of media for the bacterial culture

Nutrient Agar CM0003 from Oxiod medium was used for the cultivation of Bacteria. Dissolved 28 grams in 1000 ml distilled water. Heated to boiling to dissolve the medium completely. Sterilized by autoclaving at 15 lbs pressure (121˚C) for 20 minutes.

Experimental design and treatment combination

The experiment was conducted in triplicate and the

data is presented as mean ± SEM in the result. The data were analyzed by ANOVA followed by LSD at 5 % level of significance using MS Excel.

Statistical analysis

The experimental design was Completely Randomized Design (CRD) with in Factorial arrangement repeated three times. The data were subjected to analysis of variance technique. Incase, the data were found significant, Least Significant Difference (LSD) Test was applied for mean comparison. A Statistical software Statistix software version 8.1 was used to data analysis (Jan *et al*., 2009)

Results and Discussion

Bacterial growth Inhibition

The mean data and ANOVA for zone of inhibition of plants, concentrations and bacterial strains regarding the zone of inhibitions are given in Table 1. The original replicated data for zone of inhibition are given appendix.

Methanol extracts of medicinal plants were tested against two bacterial strains *Bacillus subtilis and E. coli* bacteria to investigate the effects of plant crude extracts on bacterial growth. The analysis of variance Table 1 indicate that the plants crude extracts have a non-significant effect on the zone of inhibition. However, the highest zone was observed in *Corcuma longa* that is 20 mm followed by *B. lycium, P. harmala* that is 18.83 mm and 18.5 mm, respectively. The lowest was observed in *S. marianum* that is 13.91 mm. Similarly, the concentration and bacterial strains have significant effect on zone of inhibition. The interactions of plant bacteria and Concentration bacteria have non-significant effect on bacteria zone of inhibition however the interactions between plants, concentration and bacteria have significantly affected the bacteria zone of inhibition. The different plants, concentrations and bacterial strains have significantly affected the bacterial growth by producing inhibition zones. Three plants *Corcuma longa*, *Berberis lycium,* and *P. harmala* showed most effective with producing inhibition zone of diammeter 20 mm. 18.83 mm and 18.5 mm respectively. The maximum zone of inhibitions 20 mm was shown by *Corcuma longa* followed by *Berberis lycium* 18.83 mm and *P. harmala* 18.5 mm. The *S. marianum* showed the minimum inhibition zone of 13.91 mm. The inhibition zones of all the medicinal plants can be arranged in decreasing order *Corcuma longa* > *Berberis lycium*> *P. harmala* > *S. marianum*. The LSD Test applied on most effective plants reveal non-significant show that all three plants are equally effective in their ability to control bacteria growth. The highest activities of some plants may be due to presence of different chemical constituents present in the extracts. The results are also in agreement with the finding of Gupta and Singh (2018) reported that *Berberus lyceum* methanolic extracts produced 28 mm zone of inhibition against various bacterial strains. The qualitative and quantitative phytochemical analysis as reported in the literature indicates the presence of Alkaloids, Flavonoids and Phenolic compounds. Thus, we deduce that these plant metabolite have active role in crude extracts antibacterial activities. These finding are in line with (Akhtar and Mirza, 2018; Ullah *et al*., 2015) who also concluded from finding of their research on medicinal antibacterial activities against that plants possess various phyto-constituents at various level so the high and low activities due to quantities of these ingredients and antagonistic effects. The finding are also in agreement with the find (Ullah *et al*., 2017). The study concludes the importance of the reported plants against bacteria. Further investigation for finding compounds/metabolites that inhibit the growth of bacteria may help in design of nutraceutical and pharmaceutical against bacterial infections. Some of the compounds isolated reported from *B. lycium, P. harmala, S. marianum* and *C. longa* include berberine, hermaline, hermine, silymarin. The alkaloid berberine derived from *Berberus lycim* has also been reported for number of wide range of clinical application including anticancer, antidiabetic, diarrhea and anti-inflammatory activities and possess define potential as drug (Tillhon *et al*., 2012; Jabeen *et al*., 2015) The high antibacterial activities of *B. lycium* may be attributed due to the presence of berberine alkaloids. Further research into its pharmacokinetics how it controls the glucose in the patients is still unknown. Curcumin, bioactive compound derived from *C. longa* has also been reported for number pharmaceutical importance and has been reported most of biological activities of turmeric (Sahne *et al*., 2017).

The study further strengthen the traditional use the plants to cure disease and ailments. However, the traditional herbal research may further be strengthen with experimentation including research with focus active compounds isolation, characterization and biological screening against the bacteria So plant is natural factory on dispense of human being to explore is useful plants and use it for drug development

against multidrug resistance bacteria. The study further validates the traditional uses of plants for the treatment of various ailment bacterial infections and diseases. It preservation and restoration for future generation is the collective responsibility as most of these plants are extinct from its natural wild habitat due to its excessive use and human activities of urbanization.

Table 1: *Analysis of variance table for bacterial zone of inhibition.*

SOV	DF	SS	MS	F-Cal	P-Value
Plants extracts (PE)	4	386.07	96.517	1.76	0.1551
Extracts Conc. (EC)	1	516.27	516.267	9.44	0.0038
Strains (S)	1	38.40	38.400	0.70	0.4071
PExEC	4	131.07	32.767	0.60	0.6655
PExS	4	652.27	163.067	2.98	0.0303
ECxS	1	15.00	15.00	0.27	0.6034
PExECxS	4	141.67	35.417	0.65	0.6319
Error	40	2188.00	54.700		
Total	59	4068.73			

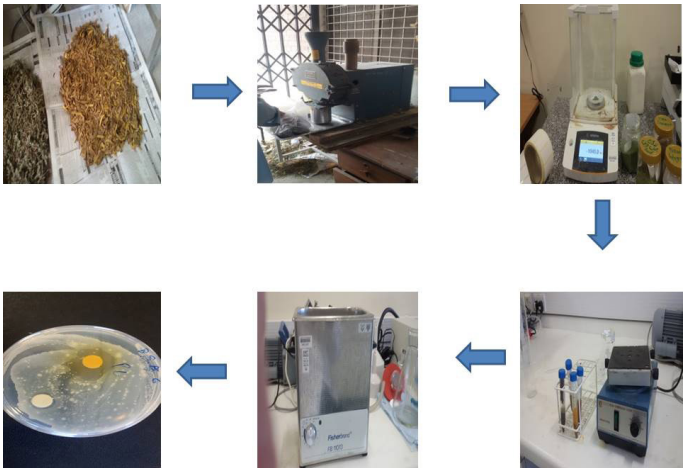

Figure 1: *Graphical Representation of Plants drying, grinding, extraction process and bioassay.*

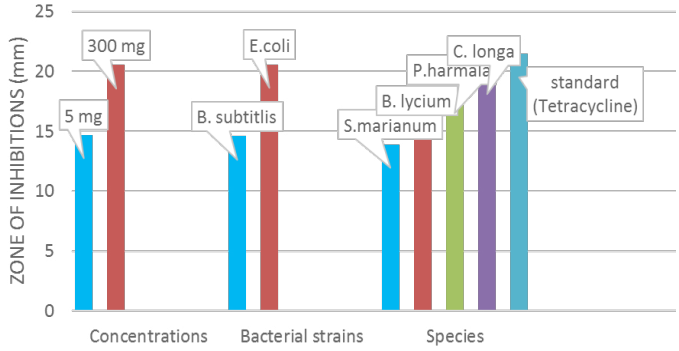

Figure 2: *Anti-bacterial activities of most effective plant extracts concentrations and bacterial strains on bacterial growth inhibition (zone of inhibition).*

The Figure 2 indicate the effect of crude plants concentration, bacterial strains and various plants crude extracts on zone of inhibition. The zone of inhibition was high for 300 mg concentrations as compare to 5 mg concentration. Similarly, the highest zone was observed for *E. coli* strains as compare *to B. Subtitlis*. In case of plants extracts the highest zone was observed for *C. longa* and lowest was noted in *S. marianum*.

These bioassays were carried out in the biosafety level 3 Microbiology Lab, Faculty of Life Sciences, University of Bradford, West Yorkshire Bradford UK. The antibacterial investigation was performed in three replicates using disc diffusion technique using Nutrient Agar Media for the growth of *Bacillus subtilis and E. coli bacteria.* The ANOVA for inhibitions zones indicates the effect of plants, bacteria strains, and concentration and their interactions. The plants crude extracts were having significant effects on the bacterial growth in bacteria culture while bacterial strains and concentrations have also significant affected the bacteria growth and zone of inhibitions was observed and recorded for plants extracts, different concentrations and bacterial strains. The various concentration and bacterial strains were having significant effect on bacteria growth at 0.05 level of Probability. The interaction effect of plant bacteria, plant concentration was non-significant and concentration bacteria interactions have significant effect on growth inhibition of Bacteria. However, the three ways interaction between plants, bacteria and concentration was non-significantly affected the bacteria growth in the culture.

The mean table indicate all the plants have showed antibacterial activities. The maximum activity was reported in *B. lycium* which seem to be most active and lowest value was recorded in *S. marianum*. The maximum inhibition zone has been reported as 20 mm for the *B. lycium* extracts and minimum extracts has been observed for *S. marianum* as 14 mm. In case of concentration the maximum growth control was notice in 300 mg (high concentration) while maximum inhibition zone was notice to be 20.53 mm for 300 mg concentrations and the minimum inhibition zone was recorded for 5 mg (low concentration) that is 14.7 mm. In case of bacterial strains, the gram positive bacteria was significantly more visible in growth by developing inhibition zone of 20 mm while minimum was recorded in gram negative

bacteria that is 14 mm. The result indicates that different plant extracts, different concentrations and different bacterial strains may affect bacterial growth inhibition differently. Therefore, further investigation into determining minimum effective concentration, plants specific activity and search for active plant metabolite would explore effective natural therapy or antibiotic for multidrug resistance pathogenic plants and human bacteria's.

Conclusions and Recommendations

The following conclusion can be drawn from the current study.

- The medicinal plants that are in traditional use as remedy for hepatic diseases confirmed to possess antibacterial activities
- The results further proved and validated that different plants crude extracts possess different level of antibacterial activity at the same concentration. The highest antibacterial activity was observed for *C. longa* followed by *B. lycium*.
- Based on the finding and synthesis of current experiment data the following are recommendation of the study.
- The medicinal plants studied in the current experiment possessed the properties to acts as antibacterial agent. These plants are recommended for herbal drug development

The antibacterial properties may be due to its phytoconstiuents. Therefore, further research investigation into determining minimum effective concentration, plants specific activity and search for active plant metabolite would explore effective natural therapy or antibiotic for multidrug resistance pathogenic plants and human bacteria's.

Novelty Statement

The study explores the uncultivated plants and highlights their medicinal importance. Some new plants such *Berberis lycium* are reported for its medicinal value in hepatic diseases for first time from swat region.

Author's Contribution

Tahseen Ullah conceived idea, abstracts, result discussion. Noor Ul Amin did overall management of article.

Conflict of interest

The authors have declared no conflict of interest.

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