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In vitro antibacterial study of *Taraxacum officinale* leaves extracts against different bacterial pathogenic strains

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Abstract
The aim of the present research was to investigate the antibacterial activity of the plant *Taraxacum officinale* leaves extracts against selected different bacterial strains. The research was done in the laboratory of Aerobiology, Department of Botany, Hazara University, Mansehra, Khyber Pakhtunkhwa, Pakistan during the month of august 2013. The extract in methanol, chloroform, and distilled water ($D.H_2O$) was used. The agar well diffusion method is used. The result of the Methanol and Chloroform extracts of *Taraxacum officinale* was found to be effective against all the tested Bacterial pathogens *P. aeruginosa, E. coli, S. aureus, Bacillus Subtilis* and *Micrococcus luteus*, while extracts in $D.H_2O$ showed no activity. Minimum inhibitory concentration (MIC), of the extracts against these bacterial strains was in the range of 0.30 mg/ml. The different phytochemical analysis result indicates the presence of secondary metabolites like Alkaloids, Tannins, and Flavonoids which may be responsible for antibacterial assay. From the results, it is concluded that extracts of *Taraxacum officinale* have potential against all bacterial pathogenic strains.

Keywords: Antibacterial activity, Agar well diffusion method*, Taraxacum officinale.*

1. Introduction
From the start medicinal plants are the main and rich source of different activity especially antimicrobial agents. Plants have been used in many countries medicinally and as a source of many potent and powerful drugs (Mahesh and Satish 2008) [1]. It has been estimated that 25% of drugs are directly or indirectly of plant origin. In the last recent years different pharmaceutical company spent lots of money and time in developing natural products extracted from plant, to produce low cost drugs that are easily affordable to the population (Doughari 2006) [2].

Medicinal plants produce a variety of compounds having very important as microbial agent and have therapeutic properties. In recent years, antimicrobial activities of plants are being increasingly reported from different countries of the world. It is concluded that plant extracts showing target sites other that those used by antibiotics will be active against drug resistant microbial pathogens. Very little information is still available on such activity of medicinal plants (Ahmad and Beg 2001) [3].

Considering the potentiality of plants as a source of drugs with reference to antimicrobial agents, a systematic study was made to screen the antibacterial activity of *Taraxacum officinale* plant.

Taraxacum officinale is an herbaceous perennial plant of the family *Asteraceae*, commonly called dandelion. The plant grows in the temperate regions of the world, on roadsides, in lawns, on distributed banks etc. *Taraxacum* leaves are used as a salad. The plant *Taraxacum officinale* has been used traditionally for poor digestion, water retention and for disease of liver including hepatitis (Dearing *et al*., 2001) [4].

The root of dandelion is a registered drug in Canada, sold principally as a diuretic. A hepatoprotective effect in mice of chemicals extracted from dandelion root has been reported (Mahesh *et al.,* 2010) [5]. Dandelion also used in herbal medicine as a mild laxative, for increasing appetite, and for improving digestion. The milky latex of the dandelion has been used as mosquito repellent (Stuart and Malcolm (1979) [6].

In the present study, the antimicrobial activity of Methanolic, chloroform and distill water extract of *Taraxacum officinale* was investigated against selected bacterial strains. The aim of the study was to explore the antibacterial activity of *Taraxacum officinale* plant.

2. Materials and methods

2.1 Collection of plant material

Fresh and healthy leaves of the disease free plant *Taraxacum officinale* were collected. The leaves were washed 3-4 times with running water and once with sterile distilled water, then air-dried in sterile blotter under shade and placed in hot air oven at a temperature of 40 ^{0}C for the period of 4-5 days till the weight become constant. Plant material was daily observed for any fungal or bacterial rotting. The dried plant material was converted to a powdered form with the help of clean grinder.

2.2 Preparation of plant extracts

The active components of the leaves materials were extracted using Methanol, Chloroform and Distill water. 30 gram of each powder leaves was soaked in 75 ml of methanol, chloroform and distill water in 250 ml sterile conical flask, incubated at 37 ^{0}C with shaking at 120 rpm for 45 minutes and kept for 23 hours. After 24 hours, the plant leaves extract was filtered rapidly through four layers of gauze, then the content was filtered through Whatman No 1 filter paper. The filtrate was then open air-dried and kept in the refrigerator until for use.

2.3 Culture and maintenance of test microorganisms for antibacterial study

Bacterial cultures of *Micrococcus luteus, Pseudomonas aeruginosa, Bacillus subtilis, Escherichia coli,* and *Staphylococcus aureus* were obtained from the Microbiology Laboratory of Microbiology Department Hazara University, Mansehra, Pakistan. All the bacterial strains were maintained on nutrient agar (NA, Hi-Media) at 37.Bacteria were inoculated in nutrient broth (NB, Hi-Media) and incubated at 37 oC for 24 hours for doing the test. Mueller- Hinton Agar (MHA, Hi-Media) and Sabouraud's Dextrose Agar (SDA) were used for antibacterial activity.

2.4 Antimicrobial activity screening

Agar-well diffusion methods were adopted for the antibacterial study for methanol, chloroform, and distil water (Ahmad and Beg, 2001) [3]. 120 microlitres of Methanolic, chloroform, and distil water extracts of the leaves were used against the test microorganisms.

2.5 Antibacterial screening by agar well diffusion method

25 ml of sterile Muller-Hinton Agar (MHA) was poured into Petri-plates and allowed to set. The plates were then seed with 0.10 ml of a 24 hour old culture and using a sterile glass rod to spread the culture, and then the plates were kept for drying. Wells were made on the plates with sterile whole puncture (8.0 mm diameter). One twenty microlitres of the plant extracts were poured into the respective wells. The plates were then incubated for the period of 24 hours at 37 ^{0}C. After 24 hours the plates were observed. The antibacterial activity of the plant extract was assessed by an inhibition zone surrounding the well and zone of inhibition was measured and expressed in millimeter.

2.6 Statistical Analysis

All the experiments were performed in triplicates. The statistical analysis was conducted by using Graph pad prism software. The data was arranged as mean and S.D (Standard deviation).

3. Result and Discussion

The present study was to investigate the antibacterial activity of the *Taraxacum officinale* in different solvent. For this purpose extracts in three solvents are used. Methanol, Chloroform, distill water. The extract was found to be effective in methanol and chloroform, but no activity was noted in D.H_2O against all tested bacterial pathogens. The minimum inhibition concentration value for the bacterial strain ranged to 0.30mg/ml. The highest activity was noted against *S. aureus* 13.0±0.5 in methanol followed by *E. coli* 14.0±0.6 in hexane. The result in methanol and chloroform was quite good while no activity was shown in D.H_2O. The MIC values of the extracts against bacterial pathogen were shown in Table 1 & Fig.1.

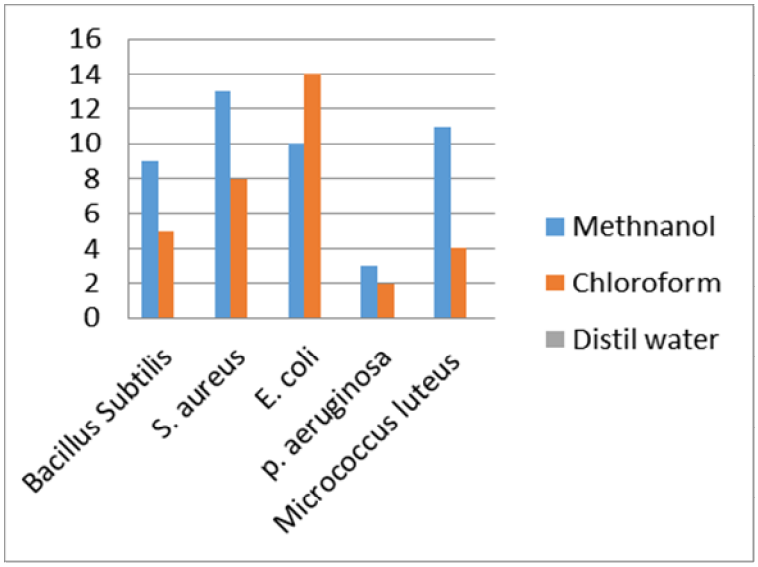

Figure 1: Extracts showing a zone of inhibition against bacterial strains

Table 1: Activity of *Taraxacum officinale* extracts against bacterial strains

Part of the plant (extracted)	Solvent	Concentration used (µl)	Zone of inhibition (mm) Mean ± S.D				
			Bacillus Subtilis	***S. aureus***	***E. coli***	***P. aeruginosa***	***Micrococcus luteus***
Leaves	Methanol	120µl	9.0±0.4	13.0±0.5	10.0±1.2	3.0±1.0	11.0±0.4
	Chloroform	120µl	5.0±0.5	8.0±0.6	14.0±0.6	2.0±0.3	4.0±0.5
	Distilled Water	120 µl	0.00	0.00	0.00	0.00	0.00

Plants from the start are important source of useful structures for the development of new chemotherapeutic agents. The first step towards this goal is the in-vitro antibacterial activity. Many reports have been available on the antiviral, antibacterial, antifungal, antihelminthic, antimolluscal and anti-inflammatory properties of plants (Mahesh and Satish 2008) [1]. Ghaima *et al.,* (2013) [7] investigated the antibacterial activity of *Taraxacum officinale* against *A.hydrophila, S. typhi, S. aureus ,B. cereus* and *E. coli* the zone of inhibitions were ranged from 14.18 mm. Some researcher had opined that the effect showed by plant extracts may be of the present secondary metabolites (Nweze *et al.,* 2004) [8]. Abdul *et al.* 2012 [9] investigated the antibacterial activity of *Taraxacum officinale* against *Staphylococcus aureus, Proteus mirabilis* and *E. coli* zone of inhibition range between 4-10mm. Dogan, 2010 [10] stated that due to these observations, it helped in identifying the active principle responsible for such activities and in the eveloping drugs for the therapeutic use in human being. Large reports are available on the exploitation of antifungal and antimicrobial property of plants for the developing commercial formulation for application in crop protection.

Due to the emergence of multi-drug resistance pathogenic bacteria as well as undesirable side effects of certain antibiotics have triggered immense interest in the search for new antimicrobial drugs of plant origin. *Pseudomonas aeruginosa* was the most resistive strain of all test bacteria used in this study. Gram negative bacteria, especially *P. aeruginosa* are frequently showed multi drug resistance to many of the antibiotics. Therefore, it is not surprising to learn that *P. aeruginosa* is the least responding bacterial strain to the tested plant extract. Oseni and Yussif, 2012 [11] stated that the *Taraxacum officinale* extracts were concentration dependent and Ethanolic extract was most active and showed good antibacterial activity and may be very useful in the discovery of new antibacterial agents.

4. Conclusion

The demonstration of activity against the bacterial strains is an indication that the plant can be a source of bioactive substances that could be of a broad spectrum of activity. Thus the broad spectrum of antibacterial activity by *Taraxacum officinale* may help to discover new chemical classes of antibiotic substances that could serve as selective agents for infectious disease chemotherapy and control.

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