



Antibacterial study of *Phyla nodiflora* Linn.

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ABSTRACT

This study describes the extraction, fractionation and antibacterial activity of *Phyla nodiflora*. The plant crude extract and all sub fractions (*n*-hexane, chloroform, ethyl acetate, *n*-butanol and aqueous) were subjected to antibacterial activities. For antibacterial activities seven bacterial strains *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Salmonella typhi*, *Staphylococcus epidermidis*, *Staphylococcus aureus* (MRSA) and *Bacillus subtilis* were used in antibacterial assay. Ethyl acetate and chloroform fractions showed excellent activity against *Bacillus subtilis*, *Staphylococcus epidermidis* and *Staphylococcus aureus*.

Key words: *Phyla nodiflora*, Extration, Antibacterial Activity.

INTRODUCTION

Medicinal plants are the 'back bone' of traditional remedy. The traditional medicine related to healing of both human and animal diseases with plant-derived preparations is considered precious information for the discovery of new antimicrobial and antifungal drugs [1]. The importance of medicinal plants as a source of active drugs emerged from the chemical profile that produces a clear physiological action on the biological system. Flavonoids, alkaloids, tannins and phenolic compounds have been established as the most important bioactive compounds of plants [2]. Medicinal plants are economically important and considerably useful. They contain some active substances which are used in the treatment of many human ailments. The plant extracts have been extracted and developed and then used against different microbes [3]. It is well known that even the most synthetic drugs have their origin from plant products [4].

Going complex world creating many pathogens that show resistance to many different kinds of drugs. These multidrug resistant pathogens species creates serious troubles and problems in different areas like residential, hospitals, industries, houses and in open air community. Now it becomes a world wide worst issue, and it counts in world wide problems. It has been reported from literature that using different kinds of antibiotics against these pathogens, some bacterial species becomes non-sensitive, decreases their sensitivity or change their sensitivity to these drugs. There are also some scientific reports about the release of endotoxins and some other secondary metabolites by these microbes, due to day by day complexity and resistivity of these microbes. Awareness about importance of medicinal plants is growing in medical communities, government, health care systems and also in common society in many developing countries. Main source of new drugs, and starting products for new drugs comes from medicinal plants [5].

Sangita et al. 2009 studied the antibacterial activity of dried aerial parts of *Phyla nodiflora* by the extraction and successive fractionation of extract using different solvents (methanol, chloroform, petroleum ether and water) in increasing order of polarity [6]. It is also evident from literature that the extract obtained from whole plant of *Phyla*

nodiflora showed promising antispasmodic activity in isolated pig ileum. The whole plant extract had no antidiabetic, antidiuretic, anticancer and antiviral activity in pig [7].

Phyla nodiflora Linn. is an essential medicinal plant belongs to family verbenaceae. It is scattered in subtropical and tropical regions. *Phyla nodiflora* is used in colic, asthma, diarrhea, bronchitis, ulcers, gonorrhea fever, knee joint pain, anti-inflammatory and antiplasmodic. The phytochemical study of plant shows that it contains flavonoids, sugar, essential oil, sterols, resins, tannins and non glucosides bitter substances [8].

In the present study we have chosen the plant *Phyla nodiflora* used in herbal medicine to determine its antibacterial property. Obviously, there are not sufficient scientific studies that confirm the antimicrobial activity of this plant. This study looks into the antimicrobial activity of this plant against some gram positive and gram negative pathogenic microorganism that causes the most common infectious diseases in communities of Khyber Pakhtunkhwa, Pakistan.

EXPERIMENTAL SECTION

Collection of sample

Phyla nodiflora plant was collected in May-June 2011 from Takht Bhai, District Mardan, Khyber Pakhtunkhwa Pakistan in flowering season. The plant was identified by plant taxonomist Mr. Nisar Ahmad, Department of Botany, Kohat University of Science & Technology (KUST), Kohat, Khyber Pakhtunkhwa Pakistan. The whole plant was washed, air dried and then crushed to coarse powder.

Preparation of crude extract and fractions

The shade dried plant of *Phyla nodiflora* was chopped and soaked in ethanol for 2 weeks and then extracted three times at room temperature in the same solvent and filtered.

The ethanolic extract was evaporated under reduced pressures to obtain a gummy residue with the help of rotary evaporator. The concentrated crude residue was further suspended in water and partitioned successively with *n*-hexane, chloroform, ethyl acetate and *n*-butanol to obtain *n*-hexane soluble, chloroform soluble, ethyl acetate soluble and *n*-butanol soluble fractions respectively. The crude extract and fractions were tightly packed and stored in refrigerator at 4°C.

Antibacterial Activities

The crude plant extracts and their subsequent solvent soluble fractions were subjected to antibacterial evaluation against seven bacterial strains *E. coli*, *P. aeruginosa*, *K. pneumoniae*, *Salmonella*, *S. epidermidis*, *S. aureus*, (MRSA) and *B. subtilis*.

Media of Muller Hinton Agar (Oxoid UK) was prepared in conical flask in accordance to the directions provided by the manufacturer. The media along with petri dishes, pipette and metallic borer were sterilized in autoclave for 15 minutes at 121°C and 15 psi pressure. The media was poured into petri dishes under aseptic condition (Laminar flow hood) [9]. The stock solutions of corresponding fractions and crude extract were prepared in Dimethyl sulfoxide (DMSO) [10].

The modified method of Perez *et al.* (1990) was followed. All of the seven strains of bacteria were obtained from Department of Biotechnology, KUST, Kohat, Khyber Pakhtunkhwa, Pakistan. Nutrient Agar media was inoculated with a given bacterial culture corresponding to 10⁶ CFU/ml. Bacterial strains were spread on the solidified agar media, then 7 mm wells were punched in the agar media by using sterile metallic borer. Stock solutions of crude extract and fractions in DMSO at concentration of 20 mg/ml were prepared and 200 µl from each stock solution was added into respective wells. The petri dishes were incubated at 37°C for 24 hours and control wells containing antibiotic (Rifampin), which is a positive control, was also run side by side in the same petri dishes. After 24 hours antibacterial activities were measured by measuring the diameter of the zones of inhibition and were compared these values of zones of inhibitions with the zone of inhibition of standard drug (Rifampin). The amount of growth in each well was measured [11, 12].

RESULTS AND DISCUSSION

In this study the detail spectrum of antibacterial activities of the plant *Phyla nodiflora* were observed which are presented in table-1 and also have been shown by the figures 1-3. The anti-bacterial study was performed against seven bacterial strains i.e. *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Salmonella typhi*, *Staphylococcus epidermidis*, *Staphylococcus aureus* (MRSA) and *Bacillus subtilis*. The *n*-hexane and *n*-butanol

fractions of plant were found to be active against *Escherichia coli* and *P. Aeruginosa*. Ethyl acetate, chloroform, *n*-butanol and *n*-hexane fractions showed promising activity against *Salmonella* and *MRSA* except the crude fraction. Among the bacterial strains *MRSA* inhibition was found to be more promising (Table-1). All fractions were reasonably active against *Staphylococcus epidermidis* and *Bacillus subtilis*. These pathogens cause infections of skin and soft tissues, ear, respiratory tract, blood and urinary tract [13].

The results shown in Table-1 indicates that the non polar solvent fraction (*n*-hexane) and the polar solvent fraction (*n*-butanol) showed promising activities against almost all tested bacterial strains compared to the crude and other fractions. Except ethanol all fractions shows enhanced resistance against Methicillin resistant *Staphylococcus aureus* (*MRSA*) and *Salmonella typhi*. The crude and other solvent soluble fractions except aqueous fraction showed good activity against *Bacillus subtilis*. The aqueous, ethanol, ethyl acetate and chloroform soluble fractions were completely inactive against *E.coli* and *P. aeruginosa*. Aqueous, ethanol and chloroform fractions were also completely inactive against *K.pneumoniae*.

CONCLUSION

Our present results suggest that each fraction has variable effects in different bioassays. It is recommended that *Phyla nodiflora* is an important plant from medicinal point of view and can be a potent one for further bio-assays which would lead to the formulation of safe herbal drugs of global interests with no or least side effects.

Table 1 : Antibacterial activities of extract and fractions of *Phyla nodiflora*

Microorganism (Bacterial strains)	Zone of inhibition (mm)						Standard Drug Rifampin (30µg)
	Aqueous fraction	Ethanol fraction	Ethyl-acetate fraction	Chloroform fraction	<i>n</i> -butanol fraction	<i>n</i> -hexane fraction	
<i>E. coli</i>	0.0	0.0	0.0	0.0	6.1	3.3	25
<i>P. aeruginosa</i>	0.0	0.0	0.0	0.0	3	6	27
<i>S. typhi</i>	8.0	4.1	16.0	13	10	14	29
<i>MRSA</i>	12.0	7.8	11.0	18	15	14	27
<i>S. Epidermidis</i>	7.0	9.7	7.0	8	6	9	26
<i>K.pneumoniae</i>	0.0	0.0	5.0	0	3.5	2	25
<i>B. subtilis</i>	6.5	12.4	11.0	11	9	7	28

Figure-1: Antibacterial activities of aqueous and ethanol fractions of *Phyla nodiflora*

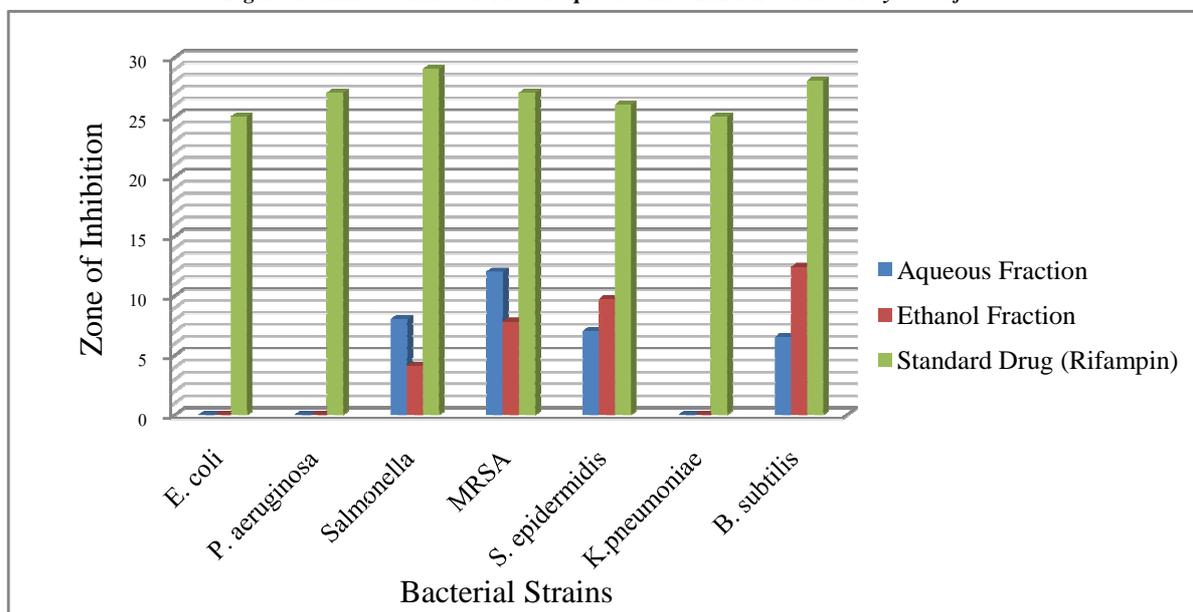
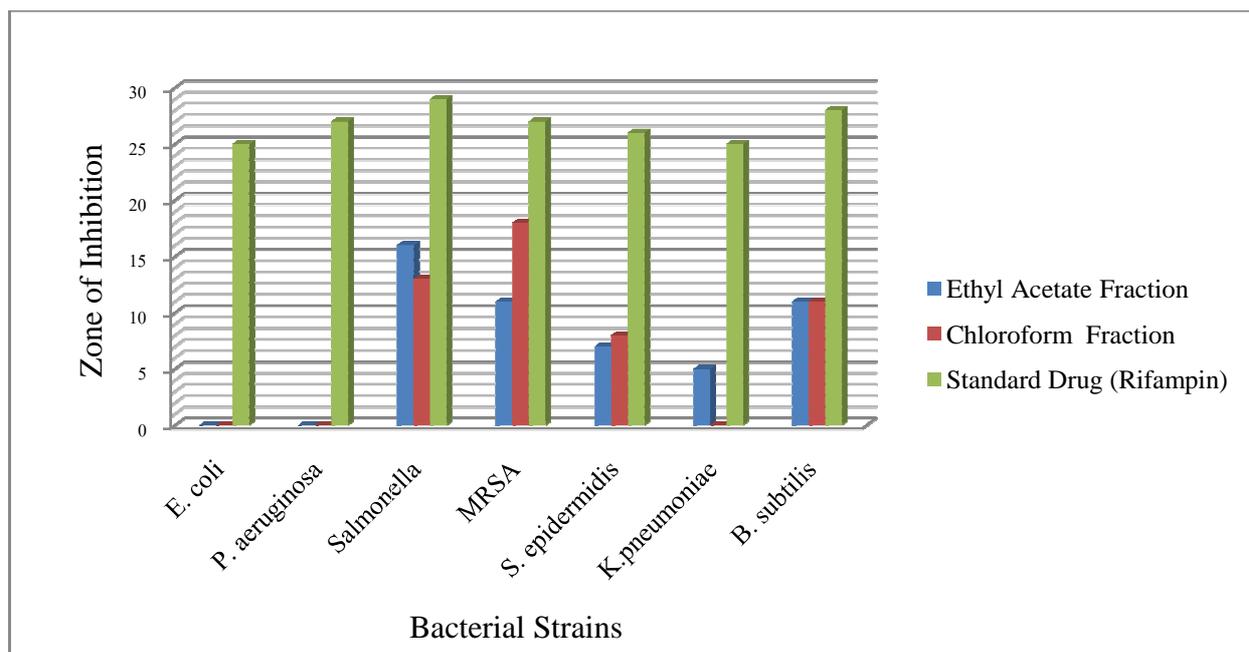
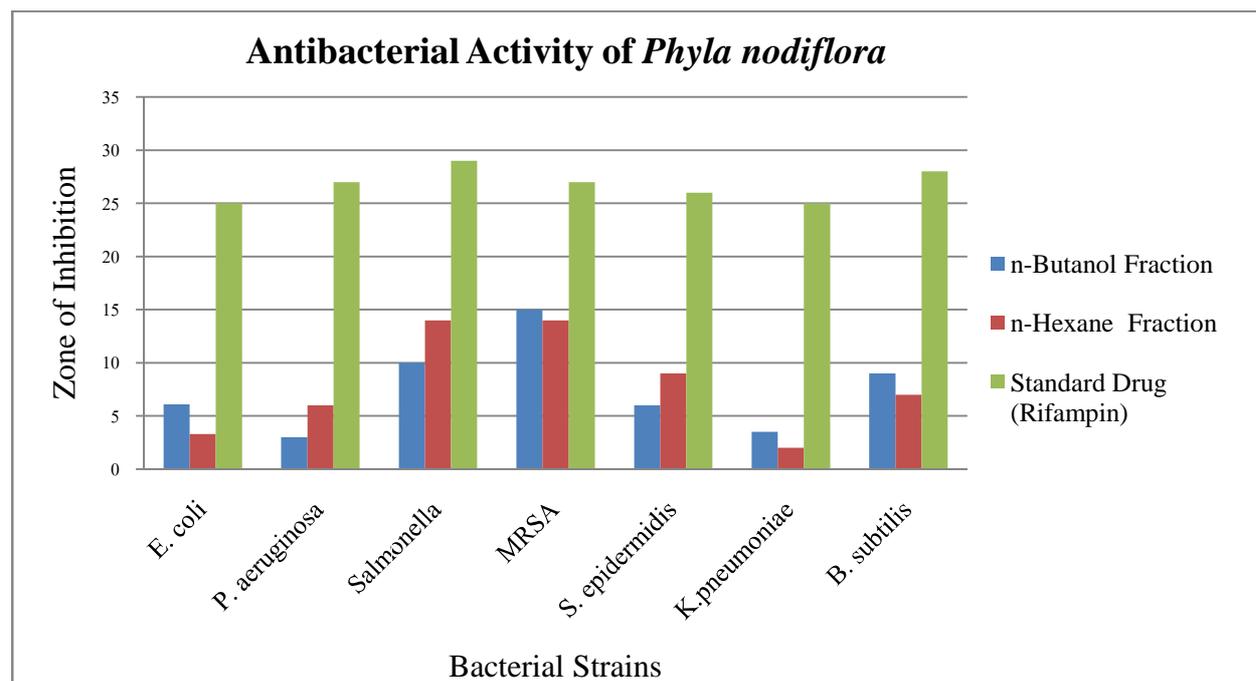


Figure-2: Antibacterial activities of ethyl acetate and chloroform fractions of *Phyla nodiflora*Figure -3: Antibacterial activities of *n*-butanol and *n*-hexane fractions of *Phyla nodiflora*

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