

## ANTIBACTERIAL ACTIVITY OF VARIOUS PHYTOCONSTITUENTS OF NEEM

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Antibacterial activity of various phytoconstituents of neem was studied to rationalize its traditional use. Neem leaves extract was carried out in order to isolate various phytoconstituents including Alkaloids, Steroids, Tannins, Glycosides, Flavonoids and Saponins. Two concentrations 50 mg/ml and 75 mg/ml of each phytoconstituent of neem and neem extract were applied against three bacterial strains i.e., *Staphylococcus aureus*, *Corynebacterium bovi* and *E.coli* by using disc diffusion method. The inhibition zones were measured in millimeter with the help of a zone reader. The data show that the inhibition zones of neem extract were greater than each of the phytoconstituents, and 75 mg/ml concentration was the more effective than 50mg/ml.

**Keywords:** Neem, *Staphylococcus aureus*, *Corynebacterium bovis*, *E. coli*, antibacterial activity

### INTRODUCTION

Neem is an evergreen tree, cultivated in various parts of the subcontinent. Every part of the tree has been used as traditional medicine for house hold remedy against various human ailments, from antiquity. Neem has been extensively used in Ayurveda, Unani and Homeopathic medicine. The Sanskrit name of the neem tree is Arishtha, meaning reliever of sickness and hence is considered as 'Sarbaroganibarini'. The tree is still regarded as village dispensary in Pakistan (Kausik, 2002).

Chemical investigation on the products of neem tree was extensively undertaken in the middle of the 20<sup>th</sup> century. The isolation of nimbin was reported as first bitter compounds isolated from neem oil, more than 135 compounds have been isolated from different parts of neem (Kausik *et al.*, 2002).

The aim of photochemical screening is to isolate various constituents of the plants for assessing their biological activity or medicinal uses. The medicinal value of plants is due to the presence of particular chemical substances that have a definite physiological action on the living system. The most important of these are alkaloids, glycosides, saponins, flavonoids, steroids, anthraquinone and tannic acid.

Alkaloids are organic nitrogenous substances. These are alkaline in nature and exhibit an extra ordinary array of pharmacological activities. Certain alkaloids act as cardiac and respiratory stimulants and are used in treatment of many types of cancer (www.entrogen.com). Glycosides are active and complex substances containing carbon, hydrogen and oxygen. They have characteristic actions on contractile forces of cardiac muscle (Hikino and Kiso, 1988). The word Saponins has been derived from Latin word 'Sapo' meaning soap. The saponins form strong insoluble complexes with body cholesterol and

thus cause its depletion by preventing its re-absorption, due to increasing its excretion, in the same way as other cholesterol lowering drugs do. Saponins also show anti-fungal, antibacterial and anti-protozoal effects (Morisaki *et al.*, 1995).

Anthraquinones are chemically known as 9, 10 dione derivatives of anthracene. Anthraquinone, glycosides are present in madder, a climbing plant and used as dye stuff (Alizarin). They possess astringent, purgative anti-inflammatory, moderate anti-tumor and bactericidal effects (Takagi *et al.*, 1980, Fatima, 1988, Feroz *et al.* 1993).

Flavonoids are widely distributed in higher plants. The flavonoids act as antioxidants which provide protection against free radicals that damage cells and tissues. A recent study indicates that flavonoids may inhibit the growth of human cancer cells in laboratory conditions. Flavonoids also support the health of entire cardiovascular system, including arterial walls (Hymete, 2005, Matsumure, 1985). Cardiac glycosides are known as cardiotoxic glycosides and are characterized by their simulative effects on heart (Rietbroch and Woodcock, 1985).

Tannins are astringent, aromatic acidic glycosides. These occur in all parts of plants including roots, stem, bark, leaves, fruits and even hairs. Tannins promote healing of wounds. These are effective in diarrhea, colitis and peptic ulcers.

The phytochemical screening has been under taken by various research workers in different countries of the world. The phytoconstituents i.e., alkaloids, glycosides, flavonoides and saponins are antibiotic principles of plants. These antibiotic principles are actually the defensive mechanism of the plants against different pathogens (Hafiza, 2000).

The present study was, therefore carried out to assess the antibacterial activity of each phytoconstituents of *Azadirachta indica* (Neem).

## MATERIALS AND METHODS

### Plant Material

The plant neem was selected for study. Its leaves were collected from Ayub Agriculture Research Institute, Jhang Road, Faisalabad. The plant materials were identified from a taxonomist, Department of Botany, University of Agriculture, Faisalabad, Pakistan.

### Native Neem Extract

The plant extract was prepared by following Osol (1975, Hymete, 2004) procedure with some modifications. Mature leaves of neem were collected and washed with distilled water. The leaves were completely dried under shady place and grind in a herbal grinder in the Laboratory of Parasitology Department. The measured amount of powdered material was soaked in alcohol (70% ethanol). The material was filtered with filter paper after one month. The filtrate was concentrated in a rotary evaporator. The remaining alcohol was evaporated in an incubator at 60°C till maximum alcohol was evaporated. The amount of extract was measured on per gram of crude powder basis.

### Phytochemical Analysis

The qualitative and quantitative estimation of various phytoconstituents i.e., Alkaloids, Saponins, Tannins, Steroids, Flavonoids and Glycosides were carried out by the method of Brain and Turner (1975).

### Microorganisms

The pathogenic strains of *E. coli*, *Corynebacterium bovis* and *Staphylococcus aureus* for antibacterial test were used. These strains were obtained from bacterial stocks, of Department of Microbiology, University of Agriculture, Faisalabad. For bacterial sensitivity test Muller Hinton (1941) agar was prepared.

### Antimicrobial Sensitivity Test

Two different concentrations (50 mg and 75 mg) of neem extract and different phytoconstituents were prepared by disc diffusion method on agar plates in triplicates following standard procedures (Bauer *et al.*, 1965).

For the antibacterial activity test glass Petri-plates of medium size (9 cm) were used. The Petri plates used for pouring was already sterilized and 15 ml of medium was allowed to sets from gel on cooling giving a layer of 2-3 mm Thickness in each plate.

The (Muller and Hinton, 1941) agar plates and potato dextrose agar plates were inoculated with inoculums of 10<sup>5</sup> size, a sterile swab is dipped in inoculums. The agar surface plates are streaked in three directions. The Whatman filter paper No. 1 with 5 mg and 75 mg of extracts and phytoconstituents were dried and

placed at agar surface with the help of sterile forceps. The Muller Hinton agar plates were incubated at 32°C for 48 hours for anti bacterial test. The antibacterial activity was then measured as indicated by clear zones of inhibition with the help of zone reader. Chloramphenicol was used as positive control for bacteria.

## RESULTS AND DISCUSSION

### Phytochemical Analysis

In subcontinent traditional medicine of neem has been used to treat malaras, hepatitis, different forms of infections and other ailments. The results of the present study have proved the scientific basis for traditional uses of neem leaves extract in the treatment of some ailments (Kausik, 2002). The phytoconstituent of neem leaves extract showed 4.1% crude alkaloids, 4.96% saponins 3% steroid, 2.5% flavonoids, 4.5% glycosides and 0.64% crude tennins respectively as shown in (Table 1). The present results were also supported by Subapriye and Nagini (2005). They isolated 140 compounds from different parts of neem. Akhtar and Farah (1987) also studied the phytochemical screening of *Melia azedareach*. They reported that fruits of *M. azedarach* contained 0.1% glycoside and 0.01% anthraquinones. Kausik (2002) reported that glycosides, flavonoids, Alkaloids, tannins, steroids were present in different parts of neem.

**Table 1. Phytochemicals and their percentage in Neem of *Azadirachta indica***

Sr. No.	Phytochemical	Result	%age in native Neem
1.	Alkaloids	+	4.1%
2.	Flavonoids	+	2.5%
3.	Crude glycosides	+	4.5%
4.	Cardiac glycosides	+	5.0%
5.	Steroid & triterpenoids	+	3.0%
6.	Tannic acid	+	0.643%
7.	Saponins	+	4.96%
8.	Anthraquinone(free)	-	
9.	Anthraquinone(bound)	-	

Phytochemical absent → -

Phytochemical present → +

### Antibacterial Activity

Antibacterial activity revealed that neem leaves extract (50 mg, 75 mg) inhibited the growth of *S. aureus*, *Corynebacterium bovis* and *E. coli* as shown in Figure 1. The results revealed that all these three strains of bacteria were sensitive against +ve control chloramphenicol. The 20 µg concentration of chloramphenicol inhibited the growth of *S. aureus*, *Corynebacterium bovis* and *E. coli* and gave good results against *S. aureus* as compared to *Corynebacterium bovis* and *E. coli*. These results were

also supported by Kher *et al.* (1984) they reported that 10% chloroform extract of neem imported inhibitory effect against *Staphylococcus aureus* and *E. coli*. Conventry and Allan (2001) also measured zones of inhibition of neem seed extract and neem azel against *B. subtilis*. Okemo *et al.* (2001) have also reported that crude extract of neem plant was very effective against *Staphylococcus aureus* and *E. coli*.

**Antibacterial activity of Phytoconstituents of Neem**

Antibacterial activity revealed that different phytoconstitunets (Crude glycosides, flavonoids, tannins, steroids, alkaloids and saponins) inhibited the growth of *S. aureus* as shown in Fig. 2. Among the phytoconstitunets crude saponins showed least anti *S. aureus* activity while crude flavonoids revealed maximum anti. *S. aureus* activity.

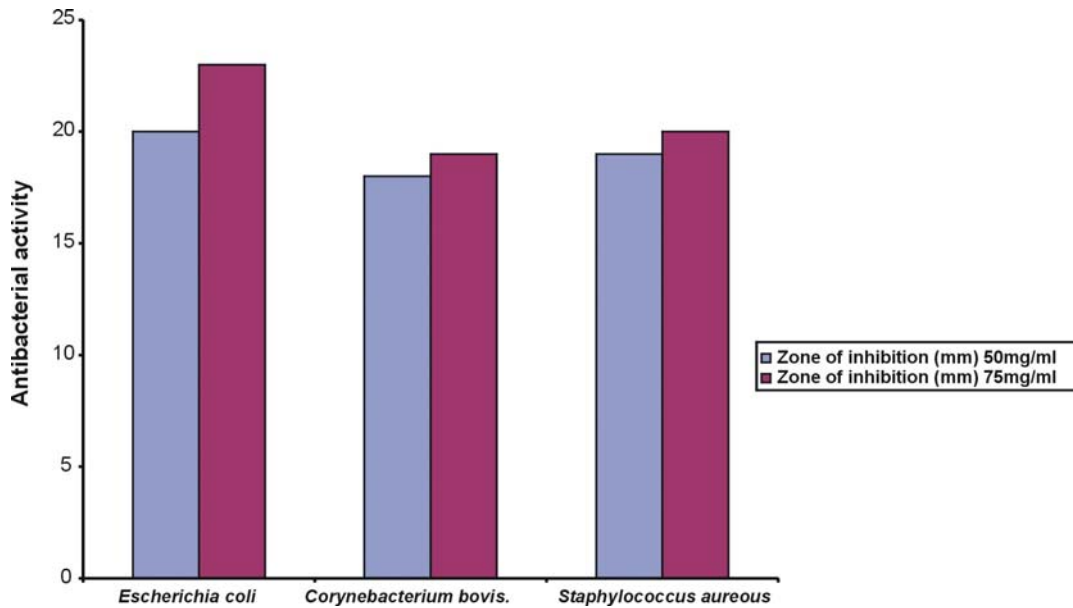


Fig 1. Antibacterial Activity of Neem Extract

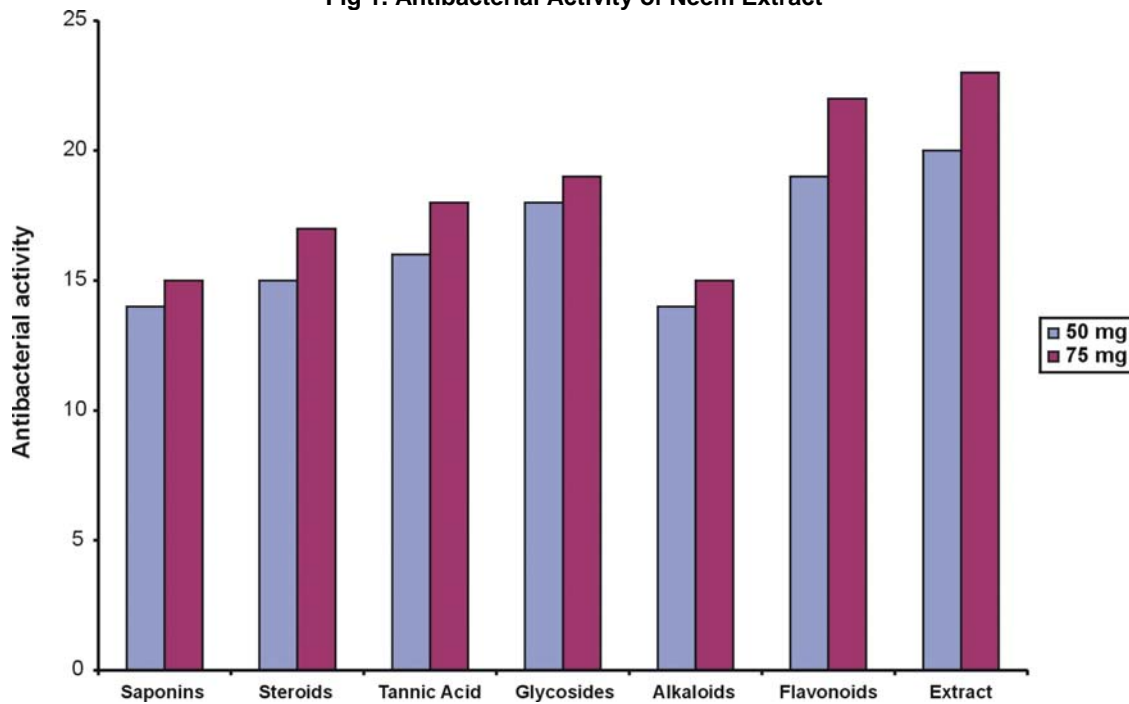


Fig. 2. Antibacterial Activity of each Phytoconstituents of Neem against *Escherichia coli*

The anti *Corynebacterium bovis* activity was also detected with different phytoconstituents. Fig. 3 showed that among phytoconstituents crude saponins were least effective while crude flavonoids were most effective against *Corynebacterium bovis*. The

glycosides flavonoids, tannins steroids, alkaloids and saponins inhibited the growth of *E. coli*. The anti *E. coli* activity was detected at 50 mg and 75 mg as shown in Fig. 4. The results were also supported by Hymete *et al.* (2005) they reported that flavonoids compounds

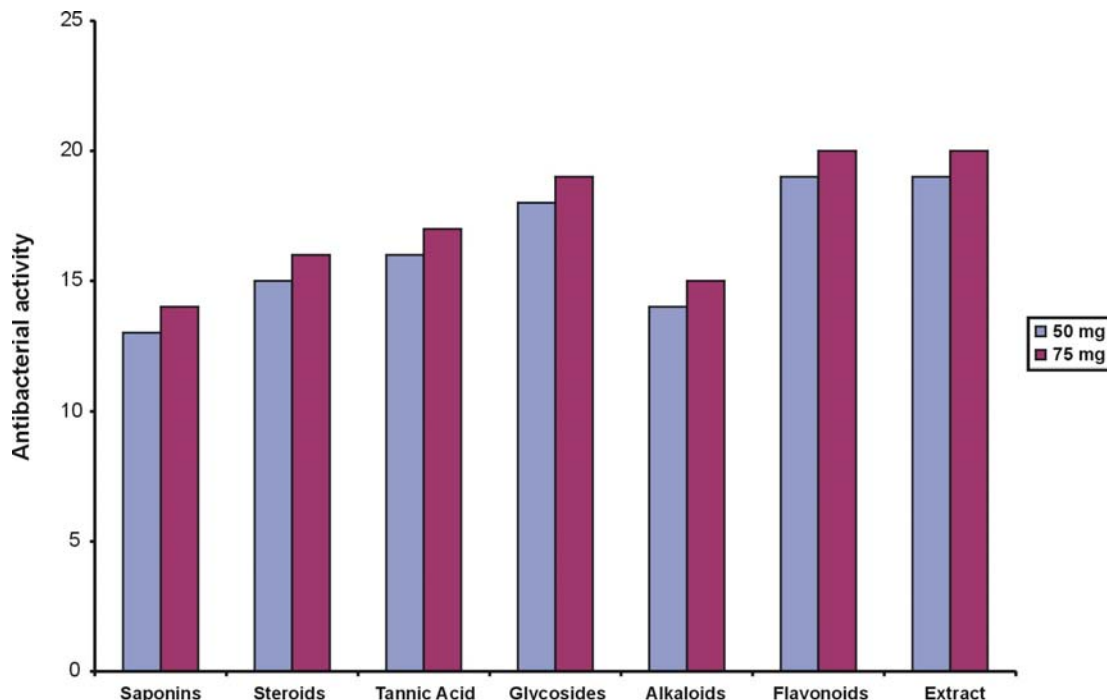


Fig. 3. Antibacterial Activity of each Phytoconstituents of Neem against *Staphylococcus aureus*

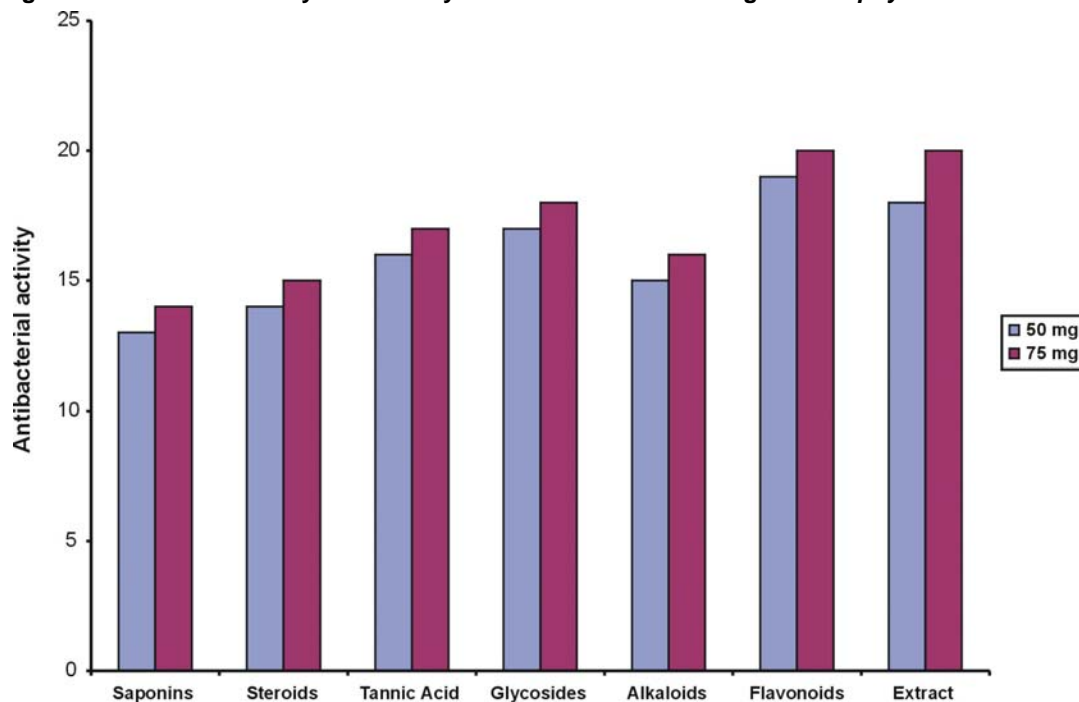


Fig. 4. Antibacterial Activity of each Phytoconstituents of Neem against *Corynebacterium bovis*

have antimicrobial activity. Hafiza *et al.* (2002) reported that crude saponins also inhibited the growth of microbes.

#### ACKNOWLEDGEMENT

The research was funded by the University of Agriculture, Faisalabad (Pakistan) under the promotion of Research scheme.

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