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# Antimicrobial Activity and Biomedical Application of *Sambucus wightiana* Phenolic Extract against Gram Positive and Gram-Negative Strains of Bacteria

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## Abstract

Increase in drug resistant pathogens has led a way for the development of new drugs by using herbal and medicinal plants extracts. In this study antimicrobial effect of methanolic and ethanolic extract of *Sambucus wightiana* was recorded positive against 9 pathogenic strains. Extracts of medicinal plant *Sambucus wightiana* possess cure for many diseases such as extract from ripe fruit cure chronic rheumatism, neuralgia and sciatica. Whole plant extract help in clearing skin and sought out skin problems and is locally used for curing fever, rheumatism and gout. The main purpose of this investigation was to evaluate antimicrobial activity of phenolic extracts of *Sambucus wightiana* against medically important bacterial strains. Antibacterial activity of whole plant's methanolic and ethanolic extract of *S. wightiana* was checked against 9 bacterial strains including gram positive and negative via agar well diffusion assay method. Antimicrobial activity has been tested against *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Vibrio cholerae*, *Enterobacter coleacae*, *Enterococcus faecalis*, *Shigella*. The result of present study suggests that extract of *Sambucus wightiana* can be used for treating diseases caused by the tested microorganisms. The ethanolic extract were more potent then methanolic against both gram negative and gram-positive strains. The activity of collected plants was investigated against bacterial strains that cause UTI, GTI, RTI allergies and Skin diseases. The collected medicinal plant was used in folk medicine for treatment of health-related problems. *E. coli* and *S. typhi* were most susceptible to methanolic extract while E faecalis was least susceptible to methanolic extract. *P. aeruginosa*, *S. typhi* and *E. coli* were most susceptible to ethanolic extract of *S. wightiana*.

**Keywords:** Antimicrobial effect; Ethanolic extract; Methanolic extract; *Sambucus wightiana*; Gram-positive bacteria; Gram-negative bacteria

## Introduction

Nature is an important source of plants that possess antimicrobial properties and biomedical importance [1]. Traditional medicine prepared from natural sources are beneficial new compounds for the development of chemotherapeutic agents. The research interest on preparation of many biological active compounds, efforts have been made to discover new antimicrobial substances which help to provide a better solution for increment in infection due to resistance of bacterial strain against antibiotics [2]. Work on antimicrobial natural active plant extract is constantly increasing due to resistant acquired by bacteria against antibiotics which has led to develop new and innovative antimicrobial agents. Many medicinal plants have a rich source of an antimicrobial substances that work as antimicrobial, antifungal activity along with that medicinal plant possess antioxidant anti cancerous and cytotoxic. Nature is filled with important sources of medicinal plants all most all plants have

therapeutic activities. Medicinal plants and herbs [3] have been curing various disorders in human from the time of stone age till now [4]. It is believed that overall, 80% of the world population believe in cure with traditional medicine and herbs for their primary health care [5].

The Earliest user of medicinal plants were in Rigveda, 1500-400 BC, Athurveda 1500 BC, Upnishada 1000-600 BC [6]. Traditional man and healers in Asia have also practiced and applied medicinal plants for curing many infectious diseases as cheap medicine as compared pharmaceutical drugs. Use of medicinal plants in Asia had a great history of human common interaction with plant, plants used for medicine have a wide range of substances that are being used and can be used to treat infectious along with that chronic diseases also [7]. Plants have certain chemical compounds that produce a specific physiological action on human body. The most important of these are Alkaloids, flavonoids, tannins, and phenol compounds [8]. We are living in the era where globalization and advancement in science is going on as population is increasing. On the other side infectious diseases are also increasing paying a vital role in high morbidity and mortality rate all over the world [9,10].

Generally, bacteria possess genetic ability to acquire and transmit resistant to therapeutic agents. Due to massive use of antibiotics in therapy bacteria have develop enormous resistance mechanism including efflux of antibiotic [10]. With bacterial resistance to many antibacterial and therapeutic agents its necessary to investigate alternative commercial medicine that pose an impact on resistant bacterial strains [11]. In the industrialized and developing countries 70% of population depends upon medicinal plants which are an important source for curing several diseases [12]. These plants are rich in antimicrobial agents and are sources of powerful and highly effective drug [13]. Herbal plants are source of synthetic and traditional medicine used to treat various diseases in India and around the globe and have long term effect [14]. Medicinal value and importance of these plants lies in the chemicals they contain for cure of diseases that produce a better physiological effect on human and animals' body [8].

World Health Organization and many other national authorities have organized the antimicrobial resistant in both medicine and agriculture as a major emerging problem of public health. The study was performed to evaluate antimicrobial activity of *Sambucus wightiana*. *Sambucus* is a genus of flowering plant of family Adoxaceae various species are commonly known as elderberry of elder. They are usually in form of 5 to 30 species of deciduous shrubs small trees and herbaceous perineal plants. Many species are widely cultivated for their ornamental leaves, flowers and fruits [15]. Being used in folk medicine in common traditional medicine elderberry had a great importance as used as medicine for curing acne and skin rash. Evidently there are very few scientific studies that confirm the antimicrobial activity of collected plant. *Sambucus* commonly grow near homesteads, it is nitrogen loving plant and not fussy about soil PH level.

This plant is commonly found in sub-tropical and temperate zones of Asia, common flora of northern areas of Pakistan and India, Kashmir. Leaves are compound; Leaflets are lanceolate, toothed, 5-9 in number. Inflorescence is terminal corymbs. Flowers are actinomorphic, minute with Calyx 5-toothedand Corolla rotate, 5-lobed and is whitish-yellow in colour. Fruit is a drupe, orange that turn black on ripening. Flowering and fruit time are from May to August and harvesting time is from September to December. Occurrence is uncommon and major part used are flower fruit and stem for different purposes. Ripe fruit extract is an excellent treatment for chronic rheumatism, neuralgia and sciatica. Whole plant work as a detoxifier help to clear skin from acne and rash and also useful in fever and cold. The paste of leaves and flower is locally used for treatment of rheumatism and gout. The current study is designed to evaluate the Antimicrobial activity of *Sambucus wightiana*.

# Material and Method

## Preparation of medicinal plant extract

The medicinal plant *Sambucus wightiana* was obtain from local hills of Kaghan valley Khyber Pakhtoonkhwa in May 2017 and identified by Head of Botany department Dr. Manzoor Hussain. The plant materials were washed under running tap water and air drain for 11 days. The dried material is processed into homogenized powder with piston and motor and stored in cool dry place.

## Ethanol extraction

By following the process of maceration 40 gram of dried powder was soaked in 300 ml of ethanol for 21 days. After 21 days it was centrifuged at 400 rpm for 15 minutes and then filtered by using what man filter paper no 4. Solvent ethanol was evaporated using water bath by adjusting temperature at 78ºC till evaporation of ethanol. Extract was collected weighed the exact weight of ethanol extract was 4.5 gm. and stored at dry cool placed in a sealed bottle.

## Methanol extraction

By following the process of maceration 40 gram of dried powder was soaked in 300 ml of ethanol for 21 days. After 21 days the it was centrifuged at 400 rpm for 15 minutes and then filtered by using what man filter paper no 4. Solvent methanol was evaporated using water bath by adjusting temperature at 68ºC till evaporation of ethanol extract was collected weighed equal to 4.75 gm and stored at dry cool placed in a sealed bottle.

## Positive and negative control

Broad spectrum antibiotic Ciprofloxacin of 250 concentrations was used as a positive control and 20% DMSO in 80% sterile water was used as a negative control [16-19].

## Determination of antimicrobial activity

Antimicrobial activity has been tested against *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa, Vibrio cholera*, *Enterobacter cloacae*, *Enterococcus faecalis*, *Shigella* [20-26].

## Culture media

The ATCC cultures were obtained from Microbiology Department and maintained on nutrient agar media all cultures were subculture weekly and subsequently incubated aerobically in incubator for 24 hrs at 37°C.

## Preparation of media

The required quantity of nutrient media 15 gm in 500 ml distilled was prepared in a sterile conical flask and sealed with cotton plug. Flask was heated to dissolve media completely and sterilized all glassware in autoclave for 15 min with temperature of 121°C according to SOP and WHO. After autoclaving poured the media in plates in a laminar cabinet providing the sterile environment in order to avoid contamination during pouring.

## Preparation of stock solution

Stock solutions of ethanol and methanol extract were prepared by using 20% DMSO in 80% sterile injection water. 2 grams of each extract was added in 5 ml of solvent (20% DMSO and sterile water) so each ml contain 400 gm/ml. Broad spectrum antibiotic ciprofloxacin with a concentration of 250 mg was used as a positive control, dissolved in dimethyl sulfoxide and water.

## Bacterial suspension

Bacterial suspension of each strain was prepared by using standard m for lens 0.5. Agar well diffusion assay is widely used to evaluate the antimicrobial activity of plant extract. For agar well diffusion assay Mueller Hinton media was used. The required quantity of Mueller Hinton media 18 gm in 500 ml was prepared in a sterile conical flask and sealed with cotton plug. Flask was heated to dissolve media completely and sterilized all glassware in autoclave for 15 min with temperature of 121°C according to SOP and WHO. After autoclaving poured the media in plates in a laminar cabinet providing the sterile environment in order to avoid contamination during pouring. The prepared agar plate surface in inoculated by a volume of bacterial inoculums by using a sterile cotton swab in a uniform order on entire agar surface. Then by using a borer a hole well in punched with a diameter of 6 mm to 8 mm aseptically. 3 well are punched in plate with a distance of 8 mm to 9 mm. By using a sterile micropipette tips exact volume of 30 µl of each extract is poured each well according to given name or serial number for identification. Different concentrations of stock solution were run 25, 50, 100 and 200 and each concentration is repeated three times. The agar plates were sealed with parafilm and incubated in incubator for 24 hrs at 37°C.

## Determination of microbial growth

Microbial growth was determined by measuring the diameter of zone of inhibition by using mm scale. p value>0.5 were considered as a standard.

# Results

The Plant *Sambucus wightiana* use in this research is belong to family *Adoxaceae* are commonly used as medical plant in different areas of world. The antimicrobial activity of Methanol extracts from *Sambucus wightiana* against various strains of bacteria are summarized in **Table 1.**

**Table 1:** Zone of Inhibition of growth (mm) showing Antimicrobial activity of methanolic extract of *Sambucus wightiana* at different concentration.

| Species | 25 mg/ml | 50 mg/ml | 100 mg/ml | 200 mg/ml |
|---|---|---|---|---|
| *S. typhi* | 24.3 mm | 32 mm | 32.3 mm | 27 mm |
| *Vibrio* | 23.6 mm | 26 mm | 31.2 mm | 28.3 mm |
| *Enterobacter* | 24 mm | 27.3 mm | 29.6 mm | 23 mm |
| *S. aureus* | 23.3 mm | 25.3 mm | 31 mm | 29.6 mm |
| *Shigella* | 23.3 mm | 26 mm | 31.6 mm | 33 mm |
| *Pseudomonas* | 21 mm | 21 mm | 34.3 mm | 38.3 mm |
| *Bacillus* | 18.3 mm | 23.6 mm | 30.6 mm | 27 mm |
| *E. coli* | 24.6 mm | 23.3 mm | 38.3 mm | 24 mm |
| *E. faecalis* | 20 mm | 21.6 mm | 17.6 mm | 32.3 mm |

The organism *Escherichia coli* (ATCC 25922) was found to be more susceptible to the methanolic extract of *Sambucus wightiana* at a Conc. 25 mg/ml give value of 24.6 mm of zone of inhibition. The *Bacillus subtillus* (ATCC 19656) was less susceptible to Methanolic extract with concentration 25 mg/ml gave the value of 18.3 mm zone of inhibition. The organism *Salmonella typhi* (ATCC 14028) was found to be more susceptible to Methanolic extract with a concentration 50 mg/ml give value of 32 mm of zone of inhibition. The *Pseudomonas aeruginosa* (ATCC 27853) was less susceptible to Methanolic extract with concentration 50 mg/ml give value of 18.3 mm zone of inhibition. The organism *Escherichia coli* (ATCC 25922) was found to be more susceptible to the *Sambucus wightiana* methanolic extract with a concentration 100 mg/ml value of 38.3 mm of zone of inhibition. The *Enterococcus faecalis* (ATCC 14506) was less susceptible to Methanolic extract with concentration 100 mg/ml value of 17.6 mm zone of inhibition. The organism *Pseudomonas aeruginosa* (ATCC 14028) was found more susceptible to Methanolic extract with a concentration 200 mg/ml value of

38.3mm of zone of inhibition. The concentration 25 mg/ml concentration 50 mg/ml concentration 100 mg/ml and concentration 200 mg/ml are summarized in **Figure 1.**

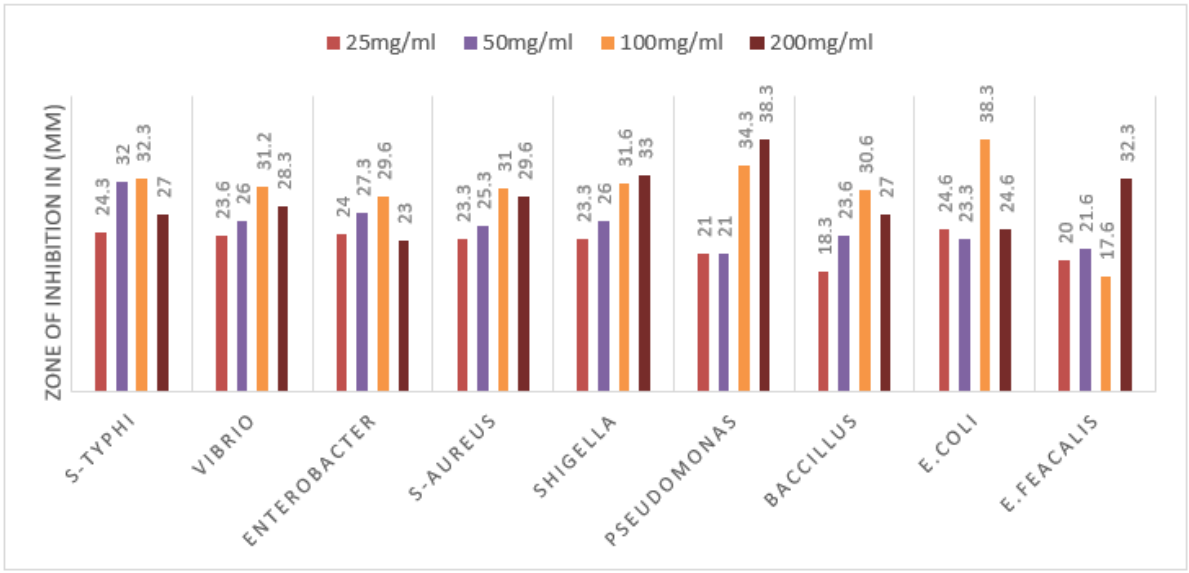


**Figure 1:** Antimicrobial activity of a plant *Sambucus wightiana* against different bacteria Strains.

The antimicrobial activity of Ethanolic extracts from *Sambucus wightiana* against various strains of bacteria are summarized in **Table 2.**

**Table 2:** Zone of Inhibition of growth (mm) showing Antimicrobial activity of Ethanolic extract of *Sambucus wightiana* at different concentration.

| Species | 25 mg/ml | 50 mg/ml | 100 mg/ml | 200 mg/ml |
|---|---|---|---|---|
| *S. typhi* | 24 mm | 30.6 mm | 39.3 mm | 26.6 mm |
| *Vibrio* | 24 mm | 27.3 mm | 26.3 mm | 37.3 mm |
| *Enterobacter* | 23 mm | 37.3 mm | 23 mm | 29.3 mm |
| *S. aureus* | 19 mm | 25 mm | 28.3 mm | 33.3 mm |
| *Shigella* | 22 mm | 26.6 mm | 34.6 mm | 35 mm |
| *Pseudomonas* | 24.6 mm | 25.3 mm | 30.6 mm | 32.6 mm |
| *Bacillus* | 23.3 mm | 23.6 mm | 22.6 mm | 31.3 mm |
| *E. coli* | 24 mm | 23.3 mm | 38 mm | 34.6 mm |
| *E. faecalis* | 26 mm | 24 mm | 32.3 mm | 33 mm |

The organism *Enterococcus faecalis* (ATCC 14506) was found to be more susceptible to the *Sambucus wightiana* Ethanolic extract with a concentration 25 mg/ml gave the value of 26 mm of zone of inhibition. The *Staphylococcus aureus* (ATCC 6538) was less susceptible to ethanol extract with concentration 25 mg/ml value of 19 mm zone of inhibition. The organism *Enterobacter coleace* was found more susceptible to Ethanol extract with a concentration 50 mg/ml value of 37.3 mm of zone of inhibition. The *Escherichia coli* (ATCC 25922) was less susceptible to ethanol extract with concentration 500 mg/ml value of 23.3 mm zone of inhibition.

The organism *Salmonella typhi* (ATCC 14028) was found to be more susceptible to the *Sambucus wightiana* Ethanol extract with a concentration 100 mg/ml value of 39.3 mm of zone of inhibition. The *Bacillus subtilis* (ATCC 19656) was less susceptible to Ethanol extract with concentration 100 mg/ml value of 17.6 mm zone of inhibition. The organism *Vibrio cholera* was found more susceptible to Ethanol extract with a concentration 200 mg/ml value of 37.3 mm of zone of inhibition. The *Salmonella typhi* (ATCC 14028) was less susceptible to Ethanol extract with concentration 200 mg/ml value of 26.6 mm zone of inhibition. The concentration 25 mg/ml concentration 50 mg/ml concentration 100 mg/ml and concentration 200 mg/ml are summarized in **Figure 2.**

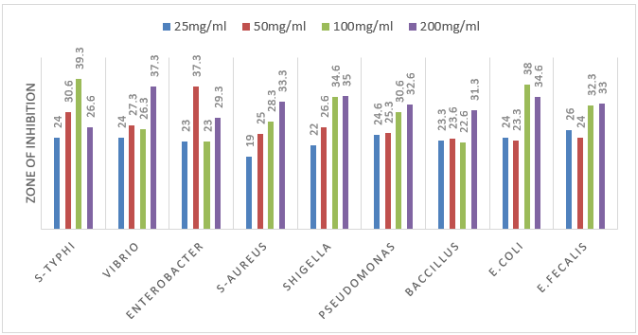


**Figure 2:** Antimicrobial activity of a plant *Sambucus wightiana*. Ethanolic extract against different bacteria Strains.

The antimicrobial activity of Antibiotic Ciprofloxacin against various strains of bacteria are summarized in **Table 3.**

**Table 3:** Zone of Inhibition of growth (mm) showing antimicrobial activity of Antibiotic Ciprofloxacin at different concentration.

| Species | 25 mg/ml | 50 mg/ml | 100 mg/ml | 200 mg/ml |
|---|---|---|---|---|
| *S. typhi* | 43 mm | 41.6 mm | 36.6 mm | 36.6 mm |
| *Vibrio* | 37.3 mm | 35 mm | 33.3 mm | 40.6 mm |
| *Enterobacter* | 44.3 mm | 33.3 mm | 26.6 mm | 35.6 mm |
| *S. aureus* | 36.6 mm | 36.6 mm | 36.6 mm | 23.6 mm |
| *Shigella* | 40 mm | 36.6 mm | 30 mm | 29.3 mm |
| *Pseudomonas* | 34 mm | 40 mm | 33.3 mm | 40.6 mm |
| *Bacillus* | 36.6 mm | 33.3 mm | 30 mm | 35 mm |
| *E. coli* | 26.6 mm | 34.6 mm | 30 mm | 19 mm |
| *E. faecalis* | 45.6 mm | 29.6 mm | 33.3 mm | 22 mm |

The organism *Enterococcus faecalis* (ATCC 14506) was found to be more susceptible to the Antibiotic Ciprofloxacin with a concentration 25 mg/ml gave value of 45.6 mm of zone of inhibition. The *Escherichia coli* (ATCC 25922) was less susceptible to Antibiotic Ciprofloxacin with concentration 25 mg/ml value of 26.6 mm zone of inhibition. The organism *Salmonella typhi* (ATCC 14028) was found more susceptible to Antibiotic Ciprofloxacin with a concentration 50 mg/ml value of 41.6 mm of zone of inhibition. The *Enterococcus faecalis* (ATCC 14506) was less susceptible to Antibiotic Ciprofloxacin with concentration 50 mg/ml value of 29.6 mm zone of inhibition. The organism *Salmonella typhi* (ATCC 14028) and *Staphylococcus aureus* (ATCC 6538) was found to be same and more susceptible to the Antibiotic Ciprofloxacin with a concentration 100 mg/ml value of 36.6 mm of zone of inhibition. The *Enterococcus faecalis* (ATCC 14506) was less susceptible to Antibiotic Ciprofloxacin with concentration 100 mg/ml value of *Enterobacter* 26.6 mm zone of inhibition. The organism *Pseudomonas aeruginosa* (ATCC 27853) and *Vibrio cholera* (ATCC) was found more susceptible to Antibiotic Ciprofloxacin with a concentration 200 mg/ml value of 40.6 mm of zone of inhibition. The *Escherichia coli* (ATCC 25922) was less susceptible to Antibiotic Ciprofloxacin with concentration 200 mg/ml value of 19 mm zone of inhibition. The concentration 25 mg/ml concentration 50 mg/ml concentration 100 mg/ml and concentration 200 mg/ml are summarized in **Figure 3.**

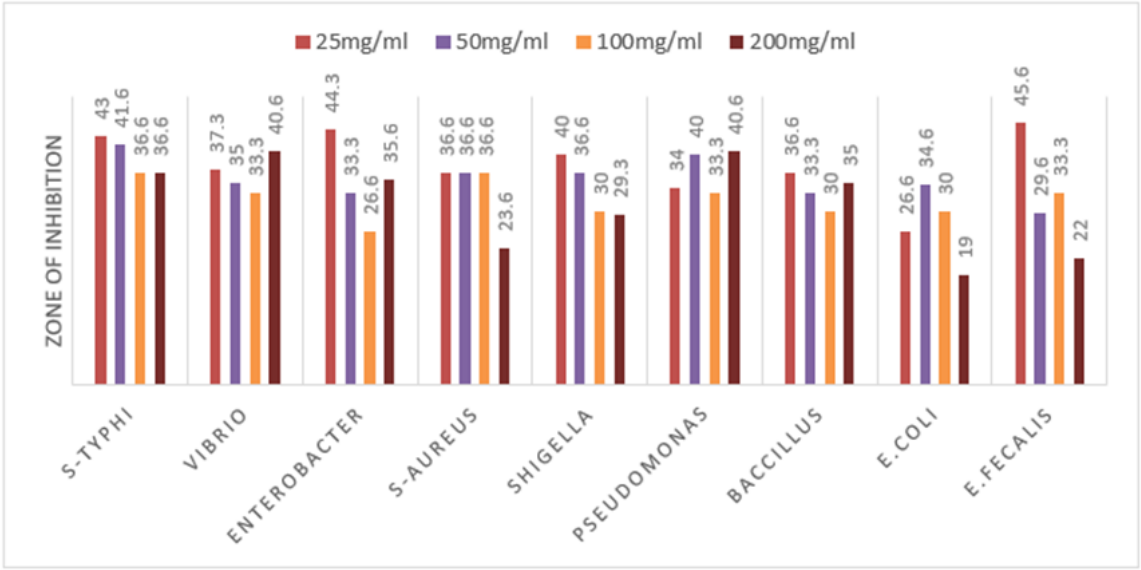


**Figure 3:** Antibiotic concentration and zone of inhibition.

## Discussion

In the present investigation antibacterial effect of *S. wightiana* nine bacterial strain was recorded including gram positive and gram-negative bacteria. Methanol extract of *S wightiana* showed maximum inhibition activity of value 24.6 mm against gram-negative *Escherichia coli* (ATCC 25922) at concentration of 25 mg/ml as compared to inhibition activity of methanol extract at concentration of 50 mg/ml which was recorded as 23.3 mm. Activity of methanolic extract against *Escherichia coli* (ATCC 25922) at concentration of 100 mg/ml was recorded as 38.3 which is greater as compared to inhibition of methanolic extract at concentration of 25 mg/ml and 50 mg/ml. Inhibition activity of methanolic extract at concentration of 200 mg/ml was recorded as 38.4 mm. As compared to methanolic extract ethanol extract of *S. wightiana* showed highest inhibition activity against *Escherichia coli* (ATCC 25922) give the mean value of 38 mm as compared to inhibition activity at 200 mg/ml which is recorded as 34.6 mm. Inhibition activity of ethanolic extract at 25 mg/ml is recorded as 24 mm and showed least inhibition activity

against *Escherichia coli* (ATCC 25922) at 50 mg/ml which is measured as 23.3 mm.

Methanolic extract of *Sambucus wightiana* showed maximum activity against gram-negative *Salmonella typhi* (ATCC 14028) at concentration of 100 mg/ml gave the mean value of 32.3 mm as compared to inhibition value at concentration of 25 mg/ml, 50 mg/ml and 200 mg/ml. Methanolic extract showed the minimum inhibition activity against *Salmonella typhi* (ATCC 14028) at concentration of 25 mg/ml gave mean value of 24.3 and inhibition activity of extract against *Salmonella typhi* (ATCC 14028) at concentration of 50 mg/ml was recorded as 26 mm which is greater than inhibition activity of extract at concentration of 25 mg/ml. Methanol extract showed inhibition activity of 27 mm at concentration of 200 mg/ml. Ethanol extract of *Sambucus wightiana* showed different inhibition activities at different concentration against *Salmonella typhi* (ATCC 14028). Highest inhibition mean value of ethanolic extract was of 39.9 mm at 100 mg/ml and showed least activity against *Salmonella typhi* (ATCC 14028) at 25 mg/ml measured as 24 mm. Inhibition activity of ethanolic extract at 50 mg/ml was recorded as 30.6 mm as compared to inhibition activity at concentration of 200 mg/ml which is calculated as 33 mm.

Methanolic extract of *Sambucus wightiana* against gram-negative *Vibrio cholera* at concentration of 100 mg/ml was recorded as highest inhibition activity with a mean value of 31.2 mm. At concentration of 25 mg/ml methanolic extract showed least inhibition value of 23.3 mm as compared to inhibition activity of methanolic extract at concentration of 50 mg/ml which is recorded as 26 mm. Inhibition activity of methanolic extract against *Vibrio cholera* at concentration of 200 mg/ml was 28.3 mm. Inhibition activity of ethanolic extract against *Salmonella typhi* (ATCC 14028) was recorded highest with mean value of 37.3 mm at concentration of 200 mg/ml as compared to inhibition value at 100 mg/ml which is recorded as 26.3 mm. Inhibition value of ethanolic extract at concentration of 25 mg/ml and 50 mg/ml were recorded as 24 mm and 27.3 mm. Inhibition activity of methanolic extract against gram negative *Enterobacter cloacae* was recorded highest at 100 mg/ml with mean value of 29.6 and least inhibition value at concentration of 200 mg/ml gave mean value of 23 mm. Inhibition activity of methanolic extract at 25 mg/ml was recorded as 24 mm and 27.3 mm at 50 mg/ml. Ethanolic extract of *Sambucus wightiana* showed different inhibition activities at different concentration against *Enterobacter cloacae*. Highest inhibition mean value of ethanolic extract was of 37.3 mm at 50 mg/ml and showed least activity against *Enterobacter cloacae* at 25 mg/ml and 100 mg/ml measured as 23 mm. Methanol extract of *Sambucus wightiana* showed different inhibition activities against gram positive *Staphylococcus aureus* (ATCC 6538) at different concentration. Maximum inhibition activity of methanolic extract was recorded at concentration of 100 mg/ml measured mean value of 31 mm. Least inhibition activity of methanolic extract against *Staphylococcus aureus* (ATCC 6538) was recorded at concentration of 25 mg/ml that is 23.3 mm. Value of inhibition activity of ethanol extract against *Staphylococcus aureus* (ATCC 6538) at concentration of 50 mg/ml was recorded as 25.3 and 29.6 at concentration of 200 mg/ml. As compared to methanol extract, ethanolic extract of *S. wightiana* showed highest inhibition activity against *Staphylococcus aureus* (ATCC 6538) give the mean value of 33.3 mm at 200 mg/ml. The measured least inhibition activity of ethanolic extract against *Staphylococcus aureus* (ATCC 6538) was recorded at concentration of 25 mg/ml that gave the value of 19 mm. The inhibition activity of ethanolic extract against *Staphylococcus aureus* (ATCC 6538) at 50 mg/ml and 100 mg/ml were recorded as 25 mm and 28.3 mm respectively.

Inhibition activity of methanolic extract against gram negative *Shigella* (ATCC 23354) was recorded highest at 200 mg/ml with mean value of 33 mm and least inhibition value at concentration of 25 mg/ml gave mean value of 23.3 mm. Inhibition activity of methanolic extract at 50 mg/ml was recorded as 26 mm and 31 mm at 100 mg/ml which is greater than inhibition value at concentration of 25 mg/ml. As compared to methanolic extract, ethanolic extract of *Sambucus wightiana* showed highest inhibition activity against *Shigella* (ATCC 23354) gave the mean value of 35 mm at 200 mg/ml. The measured least inhibition activity of ethanolic extract against *Shigella* (ATCC 23354) was recorded at concentration of 25 mg/ml that gave the value of 22 mm. The inhibition activity of ethanolic extract against *Staphylococcus aureus* (ATCC 6538) at 50 mg/ml and 100 mg/ml were recorded as 26.6 mm and 34.6 mm respectively which are better than concentration value of 25 mg/ml.

Maximum Inhibition activity of methanolic extract against gram negative *Pseudomonas aeruginosa* (ATCC 27853) was recorded at 200 mg/ml with mean value of 38.3 mm and least inhibition value was recorded at concentration of 25 mg/ml and 50 mg/ml that gave the mean value of 21 mm. Inhibition activity of methanolic extract at 100 mg/ml was recorded as 34.3 mm which is greater than inhibition value at concentration of 25 mg/ml and 50 mg/ml. On contrast to methanolic extract, ethanolic extract of *Sambucus wightiana* showed highest inhibition activity against *Pseudomonas aeruginosa* (ATCC 27853) gave the mean value of 34.6 mm at 200 mg/ml. The measured least inhibition activity of ethanolic extract against *Pseudomonas aeruginosa* (ATCC 27853) was recorded at concentration of 25 mg/ml that gave the value of 24.6 mm. The inhibition activity of ethanolic extract against *Pseudomonas aeruginosa* (ATCC 27853) at 100 mg/ml was 30.6 which is better than concentration value of 50 mg/ml that give the value of 25.3 mm.

Methanolic extract of *Sambucus wightiana* showed different inhibition activities against gram positive *Bacillus subtilis* (ATCC 19656) at different concentration. Maximum inhibition activity of ethanolic extract was recorded at concentration of 100 mg/ml measured mean value of 30.6 mm. Least inhibition activity of methanolic extract against *Bacillus subtilis* (ATCC 19656) was recorded at concentration of 25 mg/ml that is 18.3 mm. Value of inhibition activity of ethanolic extract against *Bacillus subtilis* (ATCC 19656) at concentration of 50 mg/ml was recorded as 23.6 and 27 at concentration of 200 mg/ml greater then inhibition value at concentration of 25 mg/ml.

Inhibition activity of ethanolic extract against *Bacillus subtilis* (ATCC 19656) was recorded highest with mean value of 31 mm at concentration of 200 mg/ml as compared to inhibition value at 100 mg/ml which is recorded as 22.6 mm. Inhibition value of ethanolic extract at concentration of 25 mg/ml and 50 mg/ml were recorded as 23.3 mm and 23.6 mm respectively which are better than inhibition value of concentration of 100 mg/ml.

Methanolic extract of *Sambucus wightiana* showed different inhibition activities against gram-positive *Enterococcus faecalis* (ATCC 14506) at different concentration. Maximum inhibition activity of methanolic extract was recorded at concentration of 200 mg/ml measured mean value of 32 mm. Least inhibition activity of methanolic extract against *Enterococcus faecalis* (ATCC 14506) was recorded at concentration of 100 mg/ml that is 17.6 mm. Value of inhibition activity of ethanolic extract against *Enterococcus faecalis* (ATCC 14506) at concentration of 50 mg/ml was recorded as 21.6 and 20 at concentration of 25 mg/ml greater then inhibition value at concentration of 100 mg/ml. Inhibition activity of ethanolic extract against *Enterococcus faecalis* (ATCC 14506) was recorded highest with mean value of 33 mm at concentration of 200 mg/ml as compared to inhibition value at 100 mg/ml which is recorded as 32.4 mm. Least Inhibition value of ethanolic extract was recorded at concentration of 50 mg/ml which is 24 mm and 26 mm at concentration of 24 mg/ml.

20% DMSO was used as a negative control which showed no zone of inhibition or 0 activity against bacterial strains. Broad spectrum antibiotic ciprofloxacin was as a positive control which showed maximum inhibition activity of 45.6 at concentration of 25 mg/ml against *Enterococcus faecalis* (ATCC 14506) and Least activity recorded as 26.6 mm at concentration of 25 mg/ml against *Escherichia coli* (ATCC 25922). At concentration of 50 mg/ml Ciprofloxacin showed maximum activity against *Salmonella typhi* (ATCC 14028) that is 41.6 mm and least inhibition activity against *Enterococcus faecalis* (ATCC 14506) that is 29.6 at concentration of 100mg/ml ciprofloxacin showed maximum activity against *Salmonella typhi* (ATCC 14028) and *Staphylococcus aureus* (ATCC 6538) and minimum activity against *Enterobacter coleacae*. At concentration of 200 mg/ml Ciprofloxacin showed maximum activity against *Vibrio cholera* that gives value of 40 mm and minimum activity against *Escherichia coli* (ATCC 25922) that is 19 mm.

In the present investigation antibacterial effect of *Sambucus wightiana* extracts against nine bacterial strains was recorded including gram-positive and gram-negative bacteria. Ethanolic and methanolic extract were used for checking susceptibility against bacterial strains. Methanol extract *Sambucus wightiana* showed maximum result against *Escherichia coli* (ATCC 25922), *Shigella*, *Staphylococcus aureus* (ATCC 6538), *Pseudomonas aeruginosa* (ATCC 27853), *Salmonella typhi* (ATCC 14028), *Vibrio cholerae* (ATCC 39315) and *Enterobacter coleacae* (ATCC 13047) at concentration of 100 mg/ml which is better as compare to mean inhibition value of 200 mg/ml maximum inhibition of ethanolic extract was recorded against *Shigella* (ATCC 23354), *Pseudomonas aeruginosa* (ATCC 27853) and *Enterococcus faecalis* (ATCC 14506) at concentration of 100 mg/ml, 200 mg/ml, 50 mg/ml and 25 mg/ml respectively .

In contrast with methanolic extract, ethanolic extract has more effect against gram-positive as well as gram-negative bacteria including *Enterococcus faecalis* (ATCC 14506), *Escherichia coli* (ATCC 25922), *Bacillus subtilis* (ATCC 19656), *Pseudomonas aeruginosa* (ATCC 27853), *Shigella* (ATCC 23354), *Staphylococcus aureus* (ATCC 6538) and *Vibrio cholera* at concentration of 200 mg/ml and more effective against *Staphylococcus aureus* (ATCC 6538) and *Enterobacter coleacae* at concentration of 100 mg/ml. *Staphylococcus aureus* (ATCC 6538), *Enterococcus faecalis* (ATCC 14506), *Escherichia coli* (ATCC 25922) and *Shigella* are more susceptible to ethanolic extract of *Sambucus wightiana* at concentration of 100 mg/ml and 200 mg/ml. Maximum activity of methanolic and ethanolic extract were recorded against gram-negative *Shigella* and gram-positive *Enterococcus faecalis* (ATCC 14506).

## Conclusion

The present investigation confirms that phenol extracts (methanolic and ethanolic) extracts of medicinal plant *Sambucus wightiana* have a great potential against microorganisms at different concentrations. Both plants exhibit the antibacterial activity against both gram positive and negative strains of bacteria, includes Gram-negative *E. coli*, *S. typhi*, *V. cholerae*, *Shigella*, *P. aureginosa*, *Enterobacter cloacae* and Gram-positive *S. aureus*, *B. subtillus*, *E. faecalis*, However both plants have a great potential against gram positive and negative but inhibition activity of ethanolic was recorded maximum than methanolic extract against bacterial strains.

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## Conflict of Interest

The authors report no conflicts of interest in this work.

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