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Antibacterial screening of different parts *Datura Alba Nees*

Abstract

The leaves, stem, flowers and seeds of *Datura alba Nees*. (Family: Solanaceae) were extracted successively with various organic solvents. These crude extracts were assessed for antimicrobial activities against two gram positive bacteria that were *Staphylococcus aureus, Bacillus subtilis,* two gram negative bacteria *Escherichia coli, Pseudomonas aeruginosa* by disc diffusion method. The ethanol, methanol, chloroform and acetone crude extracts of selected plant parts had significant antibacterial activities on both gram positive and gram negative bacteria. The methanolic and ethanolic extracts of leaves and flower of *D. alba* exhibited prominent activities against tested bacteria except *E. coil* used in comparison to other extracts which had moderate activity against all the tested bacteria. The antibacterial activities of the crude extracts of the selected plant parts were more active against gram positive bacteria than gram negative bacteria. The standard reference antibiotics, Erythromycin (5µg), Tetracycline (10µg) and Penicillin (10µg) were used as positive control.

Keywords: antibacterial, *datura alba*, disc diffusion, erythromycin, tetracycline, penicillin hydrophobia, epilepsy, convulsion, syphilis, hemorrhoids

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Musfara Anjum,[1] Muhammad Altaf Hussain[2]
[1]Department of Botany, Mirpur University of Science and Technology (MUST), Pakistan
[2]Department of Biotechnology, Mirpur University of Science and Technology, Pakistan

Correspondence: Muhammad Altaf Hussain, Department of Biotechnology, Mirpur University of Science and Technology, Mirpur-10250, Azad Kashmir, Pakistan, Tel +923465158668, Email altaf.bot@must.edu.pk

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Introduction

The use of plant and its products has a long history that began with folk medicine and through the years has been incorporated into traditional and allopathic medicine. Since antiquity, many plants species reported to have pharmacological properties as they are known to posses various secondary metabolites like glycosides, saponins, flavonoids, steroids, tannins, alkaloids, trepans which are therefore, should be utilized to combat the disease causing pathogens.[1]

With the advancement in Science and Technology, remarkable progress has been made in the field of medicine with the discoveries of many natural and synthetic drugs. Antibiotics are undeniably one of the most important therapeutic discoveries of the 20th century that had effectiveness against serious bacterial infections.[2] With the continuous use of antibiotics microorganism have become resistant. In addition to this problem, antibiotics are sometimes associated with adverse effects on host which include hypersensitivity, immunosuppressant and allergic reactions.[3] This has created immense clinical problems in the treatment of infectious diseases.[4]

Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases; one approach is to screen local medicinal plants for possible antimicrobial properties. Plant materials remain an important recourse to combat serious diseases in the world.[5]

Plants are known to produce a variety of compounds to protect themselves against a variety of pathogens. It is expected that plant extracts showing target sites other than those used by antibiotics will be active against drug resistant pathogens.[6] Medicinal plants have been used as traditional treatments for numerous human diseases for thousands of years and in many parts of the world. Hence, researchers have recently paid attention to safer Phytomedicine and biologically active compounds isolated from plant species used in herbal medicines with acceptable therapeutic index for the development of novel drugs.[7,8]

Datura alba is traditionally been used for the treatment of asthma, healing potential of burn wounds, muscle spasm, whooping cough, hemorrhoids and skin ulcers, hydrophobia, epilepsy, convulsion, syphilis, inflammation of the breasts, smallpox, mumps and leprosy etc. Various scientific studies reported the antibacterial activity.[9]

After scrutiny of published literature showing its medicinal importance, the present protocol has been outlined regarding the antimicrobial activity on these selected plant using different extracts. It is in view of this, that the present research was set up to evaluate the antimicrobial activity of *D. alba*, using different plant extractions against selected pathogenic bacteria.

Materials and methods

The plant material was collected from Bhimber Azad Kashmir. The leaves, stem, seeds and flowers were collected and dried carefully under shade and then homogenized to fine powder and stored in airtight bottles separately.

Extraction of plant material

The 25g portion of each dried powdered plant part material was soaked separately in 125ml Acetone, Chloroform, Methanol and Ethanol. The extraction was carried out by maceration for 7days in each solvent at room temperature (25±2˚C). The solvents extracted material was filtered in separate beakers.[10] All extracts were then dried at room temperature. The dried ethanolic, methanolic, acetone and chloroform extracts were then dissolved in their respective solvents in a proportion of 100 mg/ml. The concentration of reference antibiotics that were Erythromycin 5µg, Tetracycline 10µg and Penicillin 10µg.

The microorganisms viz gram positive bacteria i.e. *Staphylococcus aureus*, *Bacillus subtilis,* gram negative bacteria i.e *Escherichia*

coli, Pseudomonas aeruginosa were used to assess the antibacterial properties of different extracts of the selected plant parts.

Antimicrobial assay

A 24h old culture of each bacterium. The slants were prepared in test tube. The nutrient agar medium was used for bacterial growth. *In vitro* antibacterial screening was performed by disc diffusion method as described by Vander et al.[11] The sterilized nutrient agar medium when temperature reached between 40 and 45˚C was poured in the petri dishes containing bacterial suspension. Two series of experiments were conducted. In first crude extracts were tested for their antibacterial activity against already mentioned bacteria. In the second series of experiment, antibiotic discs were prepared from the dilution of commercially available standard reference antibiotics, that is, Erythromycin, Tetracycline and Penicillin were placed on the top of the medium in the centre of petri dishes by following the disc diffusion method.[11]

The purpose of this experiment set was to compare the antimicrobial activity of the standard reference antibiotics with that of the solvent extracts of leaves, stem, seeds and flowers of *D. alba*. The plates containing bacterial culture were incubated at 37°C for 24h. After the incubation time, all the plates were examined for the presence of inhibition as a property of antibacterial activity.

Results and discussion

Medicinal plants represent a rich source of antimicrobial agents. Plants are used medicinally in different countries and are a source of many potent and powerful drugs. Recent years, although technology and medicine have developed extensively, due to the decrease in natural richness made it obligatory to use natural products for many goals. For these reasons, like in other countries, in Pakistan, *Datura alba* is used for the treatment of various diseases.

In this study, the antimicrobial influence of acetone, methanol, ethanol and chloroform extracts of *D. alba* leaves, stem, seeds and flower were determined. This plant is known to have healing properties and is used for treatment of various diseases in people. The results of the antimicrobial screening of different solvents crude extracts of leaves, stem, seeds and flower of *D. alba* against four bacteria were presented in (Table 1-3) (Figure 1-6).

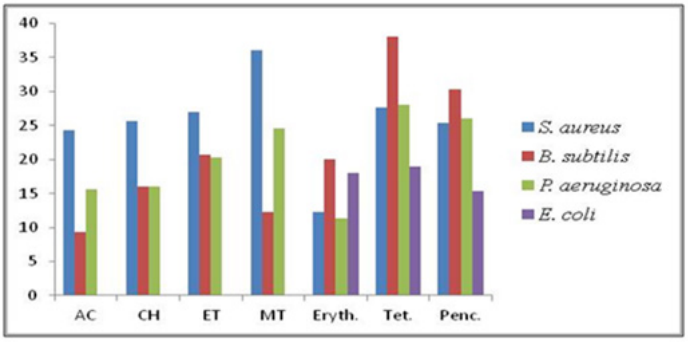

Figure 1 Antibacterial activity of leaves of *Datura alba* nees

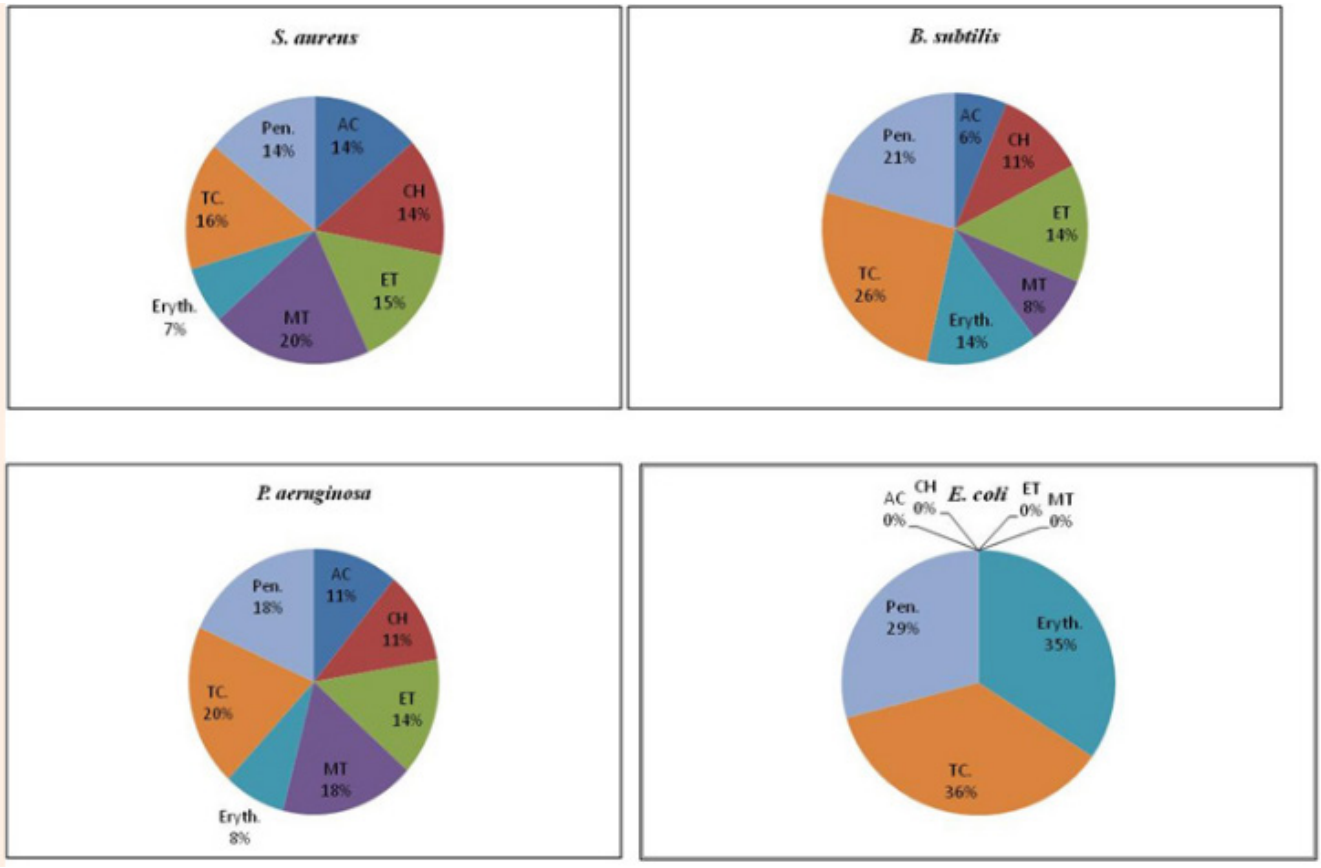

Figure 2 Pie diagram showing percentage zones of inhibition of leaves

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Table 1 Antibacterial Activity of Leaves of *Datura Alba* Nees. Concentration of Crude Extracts 100mg/Ml, Erythromycin 5µg/Ml, Tetracycline 10µg/Ml and Penicillin 10µg/Ml.

Zone of inhibition (Mm)±standard error means								
S. No	**Strains**	**AC**	**CH**	**ET**	**MT**	**Eryth.**	**Tet.**	**Penc.**
1.	*S. aureus*	24.33±0.57	25.66±0.57	27.00±1.00	36.00±0.00	12.33±0.57	27.66±0.57	25.33±0.57
2.	*B. subtilis*	9.33±0.57	16.00±1.00	20.66±0.57	12.33±0.57	20.00±0.00	38.00±0.00	30.33±0.57
3.	*P. aeruginosa*	15.66±0.57	16.00±0.00	20.33±0.57	24.66±0.57	11.33±0.57	28.00±1.00	26.00±0.00
4.	*E. coli*	_	_	_	_	18.00±1.00	19.00±0.00	15.33±0.57

AC, acetone; CH, chloroform; ET, ethanol; MT, methanol; Eryth, erythromycin; Tet, tetracycline; Penc, penicillin

Table 2 Antibacterial Activity of Flower of *Datura Alba* Nees. Concentration of crude extracts 100mg/ml

Zone of inhibition (Mm)±Standard error means								
S. no.	**Strains**	**AC**	**CH**	**ET**	**MT**	**Eryth.**	**Tet.**	**Penc.**
1.	*S. aureus*	21.00±1.00	23.66±0.57	30.33±0.57	24.00±0.00	12.33±0.57	27.66±0.57	25.33±0.57
2.	*B. subtilis*	12.66±0.57	18.00±0.00	24.33±0.57	21.66±0.57	20.00±0.00	38.00±0.00	30.33±0.57
3.	*P. aeruginosa*	14.33±0.57	19.33±0.57	20.00±0.00	20.66±0.57	11.33±0.57	28.00±1.00	26.00±0.00
4.	*E. coli*	_	_	_	_	18.00±1.00	19.00±0.00	15.33±0.57

AC, acetone; CH, chloroform; ET, ethanol; MT, methanol; Eryth, erythromycin; Tet, tetracycline; Penc, penicillin

Table 3 Antibacterial Activity of Seed of *Datura Alba* Nees. Concentration of crude extracts 100mg/ml

Zone of Inhibition (Mm) ± Standard Error Means								
S. No.	**Strains**	**Ac**	**Ch**	**Et**	**Mt**	**Eryth.**	**Tet.**	**Penc.**
1.	*S. Aureus*	_	_	15.33±0.57	_	12.33±0.57	27.66±0.57	25.33±0.57
2.	*B. Subtilis*	_	_	_	_	20.00±0.00	38.00±0.00	30.33±0.57
3.	*P. Aeruginosa*	_	_	11.66±0.57	12.33±0.57	11.33±0.57	28.00±1.00	26.00±0.00
4.	*E. Coli*	_	_	_	_	18.00±1.00	19.00±0.00	15.33±0.57

AC, acetone; CH, chloroform; ET, ethanol; MT, methanol; Eryth, erythromycin; Tet, tetracycline; Penc, penicillin

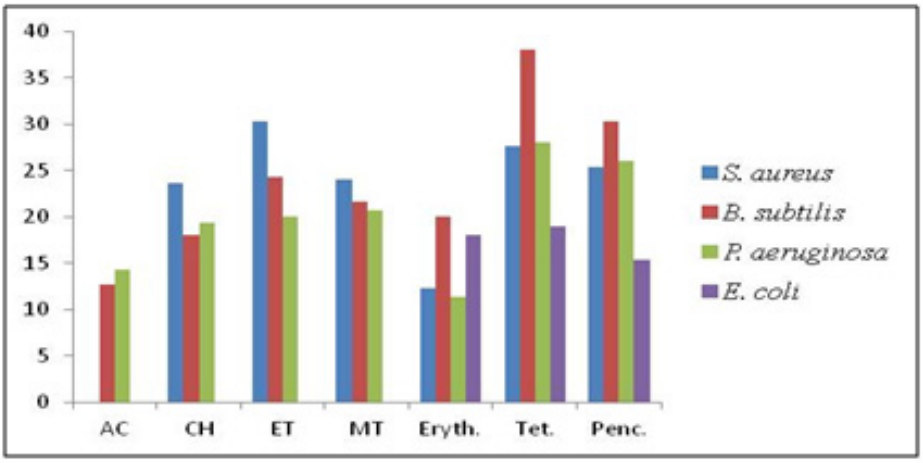

Figure 3 Antibacterial activity of flower *Datura alba Nees*

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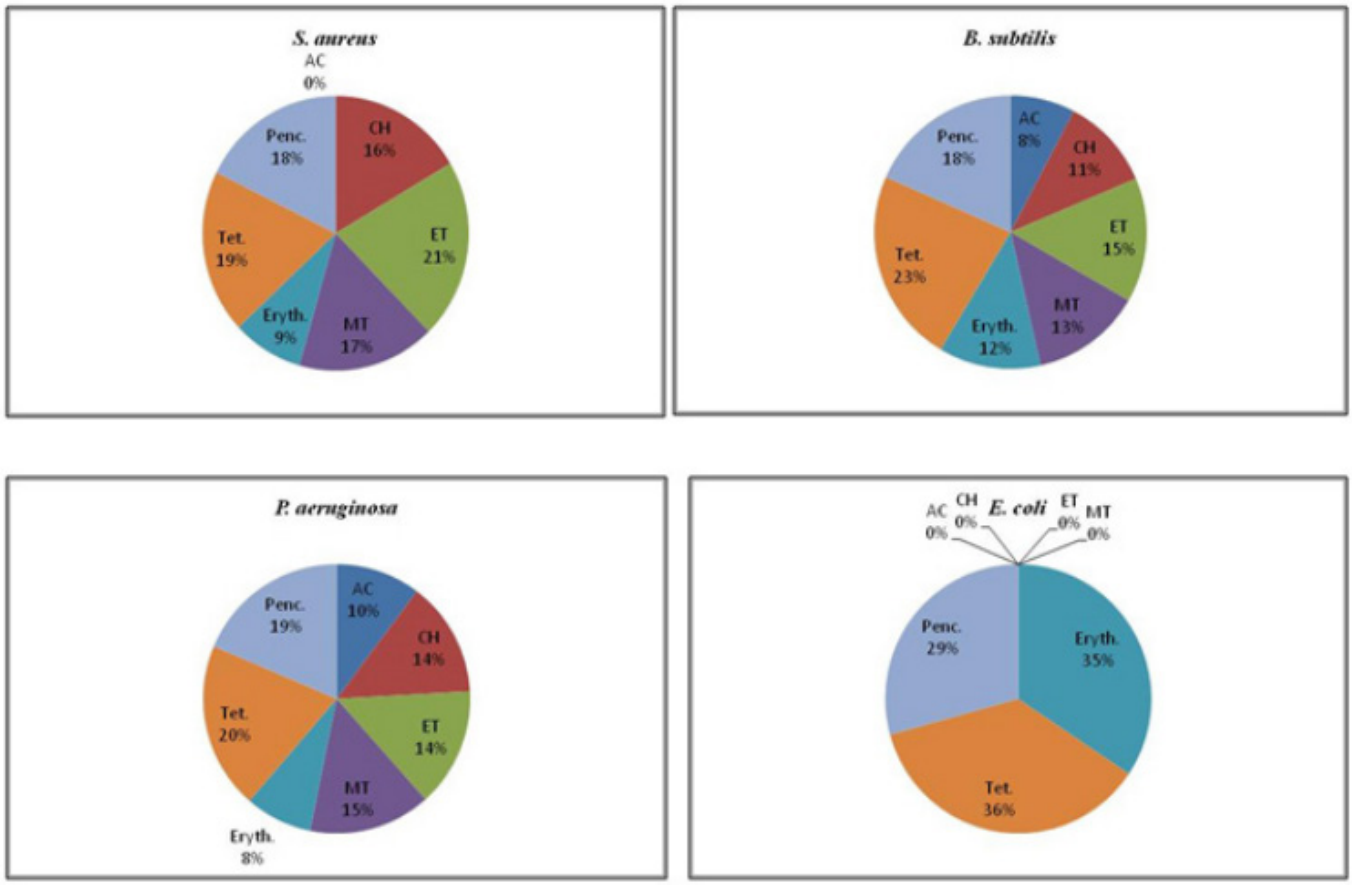

Figure 4 Pie Diagram showing percentage zones of inhibition of flowers extracts

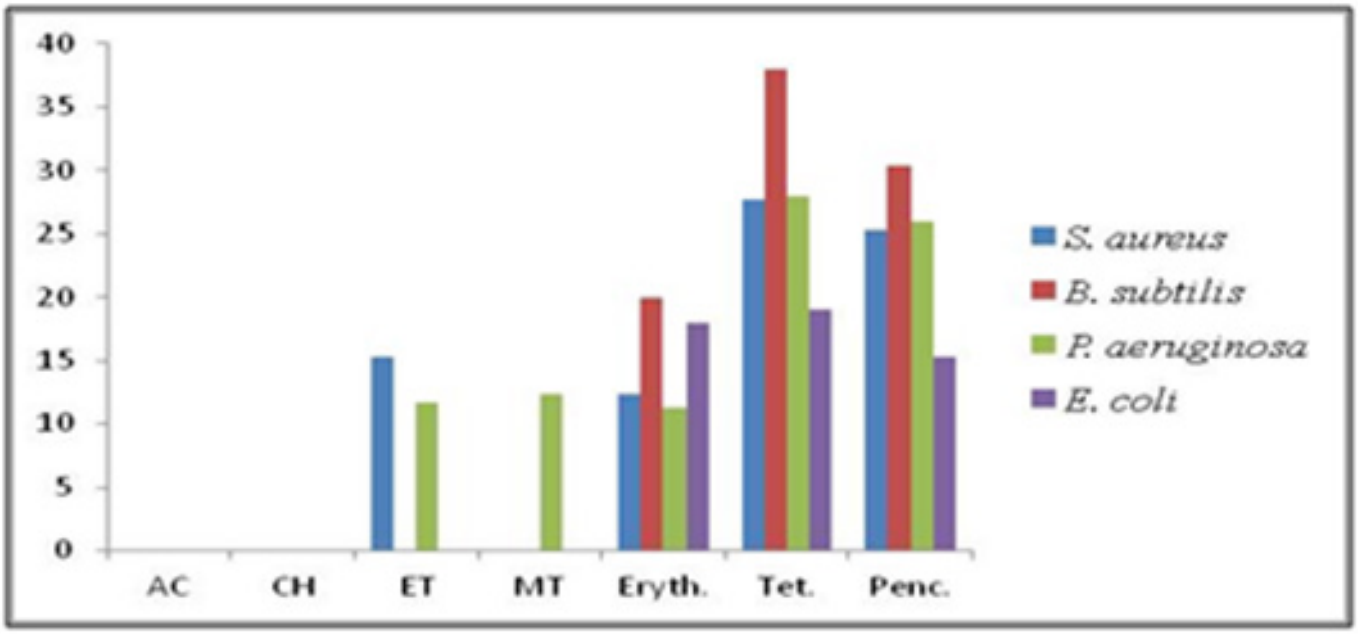

Figure 5 Antibacterial activity of seed of *datura alba* nees

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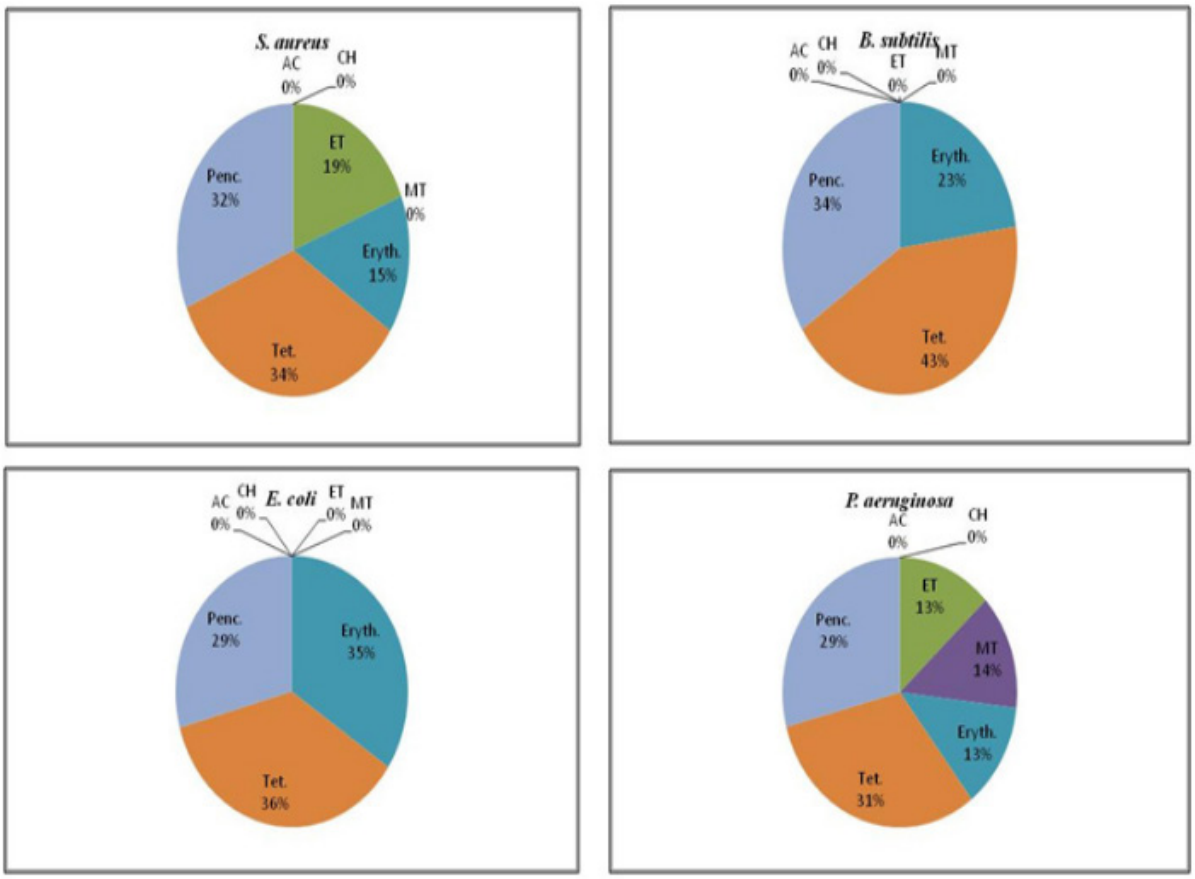

Figure 6 Pie diagram showing percentage zones of inhibition of seeds

It is evident from the results that all leaves extracts showed some activity against selected bacteria, the methanolic leaves extracts of *D. alba* showed maximum activity against *P. aeruginosa, S. aureus* and *B. subtilis* that is 24.66±0.57, 36.00±0.00 and 12.33±0.57mm, respectively while leaves showed no activity against *E. coli* (Table 1) (Figure 1,2). Ethanolic extract of leaves also showed maximum activity against *P. aeruginosa, S. aureus* and *B. subtilis*, which is 20.33±0.57, 27.00±1.00 and 20.66±0.57 respectively while leaves extract showed no activity against *E. coli*. The acetone and chloroforms extracts also showed appreciable activity against *P. aeruginosa, S. aureus* and *B. subtilis* which were given in Table 1, Figure1, Figure 2.

Table 2, Figure 3, Figure 4 showed that Flower extracts also have some activity against selected bacteria. The maximum activity showed by ethanolic extract against *P. aeruginosa, S. aureus* and *B. subtilis* that is 20.00±0.00, 30.33±0.57 and 24.33±0.57mm, respectively while flower showed no activity against *E. coli*. Methanolic extract of flower also showed maximum activity as compared to other two extracts against *P. aeruginosa, S. aureus* and *B. subtilis* that were 20.66±0.57, 24.00±0.00 and 21.66±0.57mm respectively while flower extract showed no activity against *E. coli*. The acetone and chloroforms extracts showed minimum activity than the other extracts.

Table 3, Figure 5, Figure 6 revealed that seed extracts showed minimum activity against tested bacteria. Ethanolic extract of seed showed maximum activity as compared with other extracts against *P. aeruginosa* and *S. aureus*, that is 11.66±0.57 and 15.33±0.57, respectively. The other used extracts showed minimum activity against selected microbes. Table 4, Figure 7, Figure 8 showed that the Acetone extract of stem showed maximum activity against tested bacteria i.e. *S. aureus, P. aeruginosa* and *B. subtilis* that were 10.66±0.57, 10.33±0.57 and 7±0.00 respectively, and showed no activity against *E.coli.*

These results were also comparable with the previous studies of Arias et al.,[12] that the ethanolic extracts of various parts of *A. aroma* exhibited comparatively more *in vitro* antibacterial activity against Gram positive bacteria included *Enterococcus faecalis*, *Staphylococcus aureus* and *Streptococcus progenies* and Gram negative bacteria included *E. coli*, *Klebsiella pneumoniae* and *Proteus mirabilis*.

Sharma et al.,[13] describes that the extract of *Mollugo pentaphylla* at concentration of 100% exhibited high antibacterial activity against *B. subtilis* with modest activity against *S. aureus, P. aeruginosa, E. coli* and *S. flexineria.* In the present study it was observed that the ethanolic extracts of all parts of *D. alba* exhibited less or more similar zones of inhibition against *S. aureus, P. aeruginosa* and *B. subtilis* while no activity was recorded against *E. coli* (Table 1- 3) (Figure 1-6).

Verma et al.,[14] explained that the methanolic stem extract of *T. cordifolia* have bactericidal effect on tested microorganisms i.e. *E. coli, Staphylococcus aureus* and *Staphylococcus albus*. Neelam et al.,[15] described that the methanolic extract of *Cassia fistula* legumes as compared to Chloroform, petroleum ether and ethyl acetate extracts

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exhibited potent antibacterial activity against *Staphylococcus aureus, Streptococcus epidermis, E. coli* and *Klebsiella pneumonia* but in this study methanolic extracts of leaves and flower also exhibited considerable activity against all the bacteria used except *E. coli*. The largest zones of inhibition produced by the leaves extract against *S. aureus, P. aeruginosa* and *B. subtilis* were 36.00±0.00, 24.66±0.57 and 12.33±0.57mm, respectively. The largest zones of inhibition produced by methanolic extracts of flower against *S. aureus, P. aeruginosa* and *B. subtilis* were 24.00±0.00, 20.66±0.57and 21.66±0.57 mm, respectively. *E. coli* was not influenced by the extracts of leaves and flower. Seed extracts were not showed efficient activity against tested microbes as compared with other part extracts (Table 1-3) (Figure 1-6).

The antibiotic Penicillin and Tetracycline showed high activity against all bacteria used. The zones of inhibition against *S. aureus, P. aeruginosa*, *B. subtilis* and *E. coli* were mentioned in Table 1-3 & Figure 1-6. The results of Ohadoma et al.,[16] were similar to ours. They showed that the n-hexane, ethyl acetate and methanol extract were potentially active against tested bacteria except *E. coli*. Ethyl acetate fraction showed the highest activity against *B. subtilis, S. aureus* and *P. aeruginosa.* Present results were comparable with the previous reports but some extracts were more effective against gram positive than gram negative bacteria.

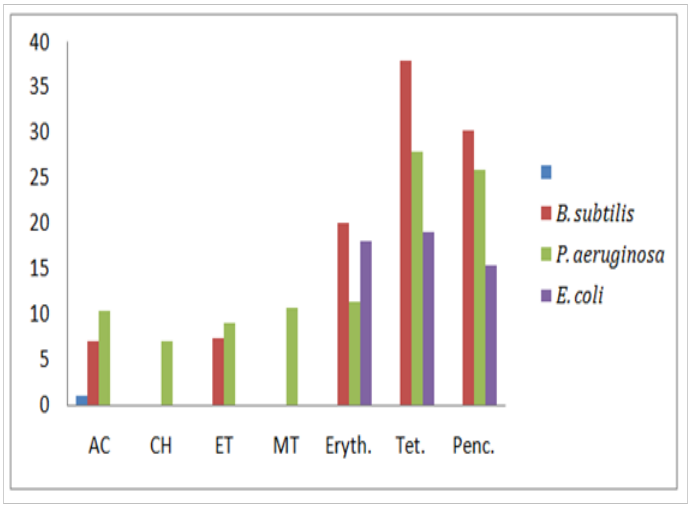

Figure 7 Antibacterial activity of stem of *Datura Alba* Nees.

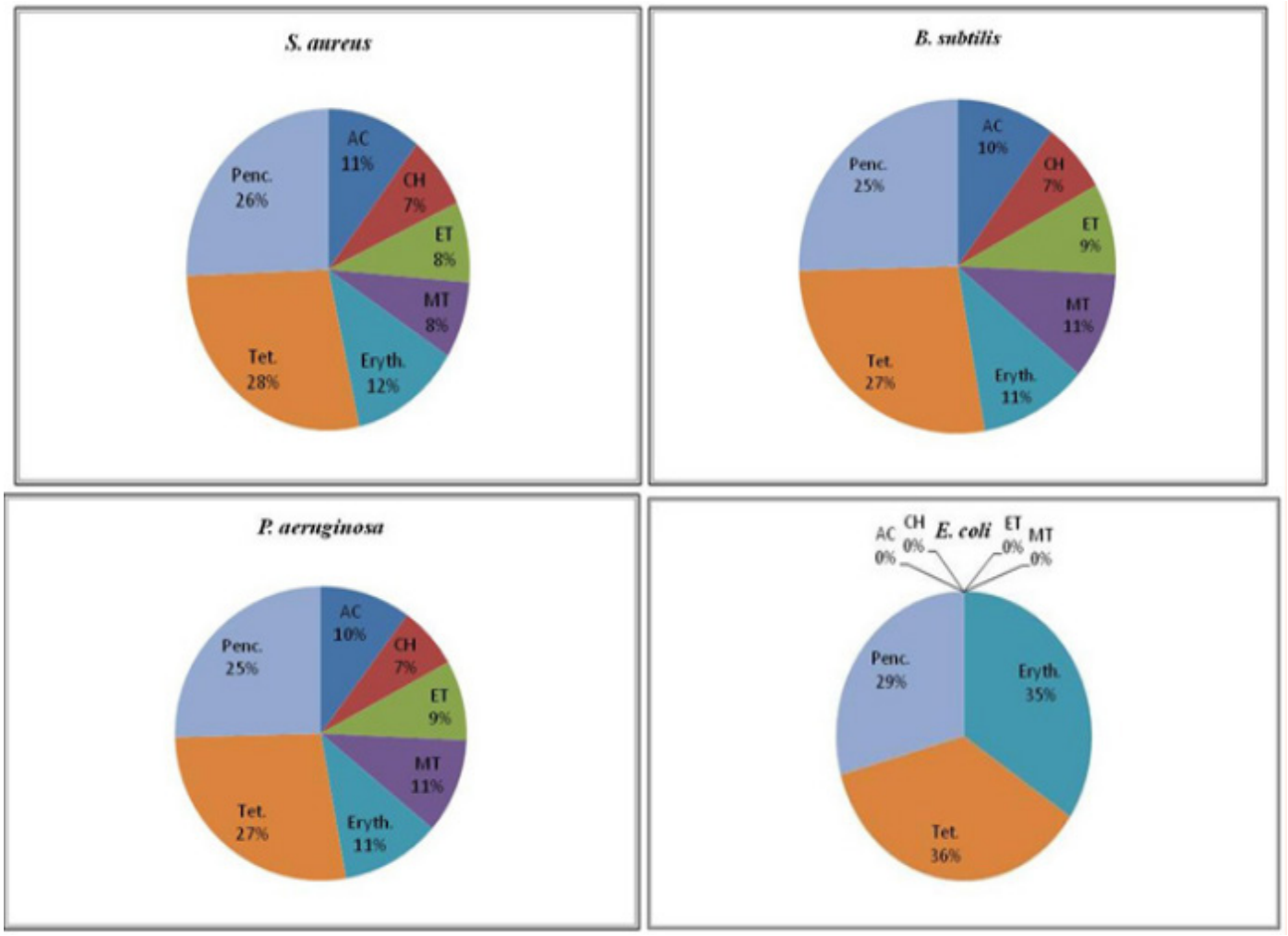

Figure 8 Pie diagram showing percentage zones of inhibition of stem.

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Table 4 Antibacterial Activity of Stem of *Datura Alba* Nees. Concentration of crude extracts 100mg/ml

Zone of inhibition (Mm) ±Standard error means								
S. No.	**Strains**	**AC**	**CH**	**ET**	**MT**	**Eryth.**	**Tet.**	**Penc.**
1	S. aureus	10.66±0.57	7.33±0.57	8±0.00	7.66±0.57	12.33±0.57	27.66±0.57	25.33±0.57
2	B. subtilis	7±0.00	_	7.33±0.57	_	20.00±0.00	38.00±0.00	30.33±0.57
3	P. aeruginosa	10.33±0.57	7±0.00	9±0.00	10.66±0.57	11.33±0.57	28.00±1.00	26.00±0.00
4	E. coli	_	_	_	_	18.00±1.00	19.00±0.00	15.33±0.57

AC, acetone; CH, chloroform; ET, ethanol; MT, methanol; Eryth, erythromycin; Tet, tetracycline; Penc, penicillin

Conclusion

Infectious diseases are one of man's oldest enemies. They continue to be a serious burden around the world, in developing and industrialized countries alike. It is said that every decade produces its own pattern of diseases. Modern medicines consist mostly of antibiotics and chemotherapeutic drugs. Still the problems of microbial resistance are growing. Multidrug resistance towards the antibiotics and their related effects has an added effect in pursuit of the use of natural drugs. Given the evidence for rapid global spread of resistance and clinical isolates, the need for discovery of new antimicrobial agents is of paramount importance. Plants are the basic source of knowledge of modern medicine. Almost all the parts of the plant, namely leaves, flowers, fruits, bark, roots, stem and seeds are known to have various medicinal properties. The trend of using natural products has increased and the active plant extracts are frequently screened for new drug discoveries and for the presence of antimicrobials and antioxidants. One such medicinal plant used in the present study is *Datura alba*. Even though the plant parts are used in traditional medicine against several disorders, especially those against microbial infections. The tested drugs (extracts of leaves and flower of *D. alba*) showed excellent antibacterial activity against tested bacteria. So it can be regarded as good natural antibiotics with considerable degree of antimicrobial activity.

As a consequence of this study, we will try to isolate pure compound, which is present in fractions showing large inhibitory activity to bacteria as well as any pharmacological or toxicological properties that such compound might have.

Acknowledgements

None.

Conflict of interest

The author declares no conflict of interest.

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