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# Phytochemical Screening and Antibacterial Activity of Solanum Trilobatum L. and Azadirachta Indica A

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**ABSTRACT**

Medicinal plants play a pivotal role in curing various human ailments. The incidence in the frequency of diseases due to pathogenic microorganisms has increased alarmingly due to the development of resistance to existing drugs and the present scenario necessitate the continuous search for new classes of antimicrobial agents preferably from natural resources. Phytochemicals are ecologically derived secondary metabolites synthesized by the plants from the primary metabolites such as carbohydrates, lipids and amino acids to protect them against environmental challenges such as UV- irradiation, extreme cold, drought, microbial attack, wound, sugar and nutrient deficiency. They often contribute to the unique odor, taste and color in plants. Diseases due to pathogenic microbes pose a great burden on human health and they have been correlated with socioeconomic, environmental, ecological factors. The threat due to infectious diseases is further intensified by the continued emergence of new and multidrug resistant microorganisms.

**Keywords:**
Pathogenic Microorganisms, Antimicrobial, Phytochemicals, Multidrug resistant.

## 1. Introduction

Nature has bestowed on us a very rich botanical wealth. Large number of diverse plants grows in different parts of the country as well as in the world. World health organization (WHO) lists more than 21,000 species of plants as medicinal plants. Medicinal plants form the backbone of traditional system of medicine in India. India is the largest producer of medicinal herbs and it is therefore known as the "Botanical garden of the world". Medicinal plants are used as pharmaceuticals, neutraceuticals, cosmetics and food supplements. The drugs are derived from plant or from leaves, stem, bark, flower and seed. Some drugs are prepared from excretory plant products such as gums, resins and latex. Medicinal plants represents a rich source from which anti microbial agents may be obtained. Plants are used medicinally as a source of many potent and powerful drugs ( Richard, 2014).

Subsequent to the serendipitous discovery of penicillin in the year 1928, antibiotics have been recognized as the only means of effective to control the pathogenesis of microorganisms. Along with the usage of new antibiotics as therapeutics, there is a developing threat of drug-resistance among the pathogenic microorganisms worldwide. Recent advances in the field of medicinal chemistry lead to the discovery of isolating the active phytochemicals from various parts of the medicinal plants for treating human infectious diseases (Bennett ,1994; Chang, 2013). Numerous structural analogs of plant secondary metabolites have been successfully generated and widely used for their pharmacological actions (Rao,2002). In present study two medicinal plants have been selected to find out phytochemicals and antibacterial activity. They are *Solanum trilobatum L. (Solanaceae)* and *Azadirachta indica A.* (Meliaceae). All infectious diseases either newly emerging or remerging represent a continued threat to humanity. Some pathogens, after a period of quiescent, are capable of acquiring features that enable them to reemerge their original or new hosts, usually in increasingly alarming proportions.

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## 2. Materials And Methods

### Collections And Preparation Of Plants Extract

Fresh plants of *Solanum trilobatum L. (Solanaceae)* and *Azadirachta indica A.* were collected in and around Chennai. Fresh plant materials were washed under running tap water, dried under shade, then ground to fine powder and stored in air tight bottles.

### Aqueous Extraction

5g of dried plant material was soaked in 100ml of water for 48 hrs with intermittent shaking. Then the extract was filtered through filter paper or 8 layers muslin cloth. Then the filtrate was collected. This filtrate was used for phytochemical screening and TLC. The filtrate was pooled and concentrated using vacuum evaporator. Then the concentrate was evaporated to dryness at 45°C. Methanol and ethanol extracts were prepared in the same way as aqueous extract. All extracts were dissolved in Dimethyl sulphoxide and methanol (1 :1 ) mixture and used for assay of antibacterial activity.

### Bacterial Culture

The leaf extracts were tested for possible antibacterial activity in the disc diffusion method using the following clinical isolates - Staphylococcus aureus, Streptococcus mutans (Gram positive), Klebsiella pneumoniae, Escherichia coli, Pseudomonas aeruginosa ( Gram negative).

### Qualitative Phytochemical Analysis

Phytochemical screening was carried out on Solanum Trilobatum L and Azadirachta Indica A using standard procedures to identify the constituents by the method of Trease and Evans (1989) and Harborne (1973).

### Qualitative Analysis Of Thin Layer Chromatography

### Tlc Plate Preparation

An inorganic substance such as silica gel is used as a stationary phase. Silica gel is made in to slurry using sodium acetate buffer. The prepared slurry is then poured on to the glass plate and spread evenly as thin flim. The plates are then dried at 80°C by keeping in an oven for 2 hours.

### Sample Application

The sample is applied using a micropipette as a spot leaving 2 or 2.5cm from the edge of the glass plate and dried. Care must be taken not to scrap the thin layer of the stationary phase while applying the sample. The plate is then placed in a chamber containing the solvent mixture (mobile phase) and developed by ascending chromatography.

### Preparation Of Inoculum

A loopful of culture is transferred to 10ml tryptic soy broth and the tubes were incubated at 37°C. Turbidity is measured visually according to Mc Farland standard. Antibacterial activity assay was done by the method by Bauer et al 1966 and Mueller and Hinton 1941.

## 3. Results And Discussion

The demand for medicinal plants is increasing worldwide due to growing recognition of natural products, being non-toxic, and more efficacies, easily available at affordable prices. Throughout

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the world, many traditional plants have been found successful for the treatment of several primary and secondary health complications. Further, most of the marketed medicines are distillations, combinations, reproductions or variations of substances that exist in nature (Ravishankar, 2000; Ravishankar, 2000).

The qualitative analysis of phytochemical screening of aqueous, methanol and ethanol leaves extract of *Solanum trilobatum L*. and *Azadirachta indica A.* Phytochemicals such as tannin, phlobatannin, saponin, flavonoid, cardiac glycoside, steroid, terpenoid, carbohydrate and amino acid was found to be present in all the leaves extract of *Solanum trilobatum L*. and *Azadirachta indica A in* table I. Table II depicts the colour of the spots of secondary metabolites which are separated by thin layer chromatography. Based on the chemical nature, the secondary metabolites are mainly classified into alkaloids, steroids, saponins, tannins, lectins, pectins, terpenoids, anthraquinones, flavonoids, glycosides and phenolic compounds (Mouhssen,2013; Amenu, 2014; Dias, 2012). Interestingly, these secondary metabolites are known to bring out significant pharmacological and beneficial effects to alleviate chronic diseases such as cancer, diabetes and cardiovascular diseases due to their antioxidant, anti-inflammatory and regulatory actions.

**Table 1:** Phytochemical Screening Of Aqueous, Methanol And Ethanol Leaves Extracts Of Solanum Trilobatum L. And Azadirachta Indica *a.*

| **PHYTOCHEMICALS** | ***SOLANUM TRILOBATUM L*** | | | ***AZADIRACHTA INDICA A*** | | |
|---|---|---|---|---|---|---|
| | **AQUEOUS** | **METHANOL** | **ETHANOL** | **AQUEOUS** | **METHANOL** | **ETHANOL** |
| Tannin | + | + | + | + | + | + |
| Phlobatannin | + | + | + | + | + | + |
| Saponin | + | + | + | + | + | + |
| Flavinoid | + | + | + | + | + | + |
| Steroid | + | + | + | + | + | + |
| Terpenoid | + | + | + | + | + | + |
| Cardiac glycoside | + | + | + | + | + | + |
| Carbohydrate | + | + | + | + | + | + |
| Aminoacid | + | + | + | + | + | + |

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"+"= Positive

**Table 2:** TLC - Separation Of Phytochemicals Of Aqueous, Methanol And Ethanol Leaves Extracts Of Solanum Trilobatum L. And Azadirachta Indica A.

| **SECONDARY METABOLITES** | **NAME OF THE TEST** | ***Solanum Trilobatum L.*** | ***Azadirachta Indica A*** |
|---|---|---|---|
| | | **COLOUR OF THE SPOT(OBSERVED)** | **COLOUR OF THE SPOT(OBSERVED)** |
| Alkaloids | Dragendorff's test | Green | Brown |
| Flavonoids | Aluminium chloride test | Blue | Green |
| Glycosides | Diphenylamine test | Green | Green |
| Phenols | Folin-ciocalteu test | Blue | Blue |
| Saponins | Iodine test | Brown | Greenish yellow |
| Sterols | Vanillin test | Brown, Pink | Green, violet, pink, yellow |
| Terpenoids | Dimethylamino benzaldehyde | purple | Greenish brown |

**Table –3:** In Vitro Antibacterial Activity Of Aqueous, Methanol And Ethanol Leaves Extracts Of Solanum Trilobatum L.

| **MICRO ORGANISMS** | **AQUEOUS** | | **METHANOL** | | **ETHANOL** | | **CONTROL** | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | **Amoxycillin** | |
| | **CONC (2mg)** | **CONC (10mg)** | **CONC (2mg)** | **CONC (10mg)** | **CONC (2mg)** | **CONC (10mg)** | **CONC (2mg)** | **CONC (10mg)** |
| | Zone of inhibition(mm) | | | | | | | |
| Staphylococcus aureus | 11 | 9 | 11 | 13 | 10 | 9 | 32 | 38 |
| Klebsiella pneumonia | 17 | 7 | 7 | 7 | 7 | 7 | 30 | 35 |
| Streptococcus mutans | 9 | 9 | 10 | 11 | 9 | 12 | 24 | 27 |
| Escherichia coli | 7 | 7 | 10 | 7 | 12 | 7 | 26 | 29 |
| Pseudomonas aeruginosa | 7 | 8 | 8 | 7 | 9 | 9 | 28 | 30 |

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**Table 4:** In Vitro Antibacterial Activity Of Aqueous, Methanol And Ethanol Leaves Extracts Of Azadirachta Indica A

| MICRO ORGANISMS | AQUEOUS | | METHANOL | | ETHANOL | | CONTROL Amoxicillin | |
|---|---|---|---|---|---|---|---|---|
| | CONC (2mg) | CONC (10mg) | CONC (2mg) | CONC (10mg) | CONC (2mg) | CONC (10mg) | CONC (2mg) | CONC (10mg) |
| | Zone of inhibition(mm) | | | | | | | |
| Staphylococcus aureus | 8 | 8 | 10 | 11 | 9 | 10 | 32 | 38 |
| Klebsiella pneumonia | 7 | 7 | 8 | 9 | 7 | 8 | 30 | 35 |
| Streptococcus mutans | 8 | 8 | 9 | 10 | 10 | 9 | 24 | 27 |
| Escherichia coli | 8 | 9 | 9 | 10 | 9 | 9 | 26 | 29 |
| Pseudomonas aeruginosa | 7 | 8 | 7 | 9 | 8 | 9 | 28 | 30 |

**Figure** 1 *IN VITRO* Antibacterial activity of aqueous, methanol **and ethanol leaves extracts of *Solanum Trilobatum L.***

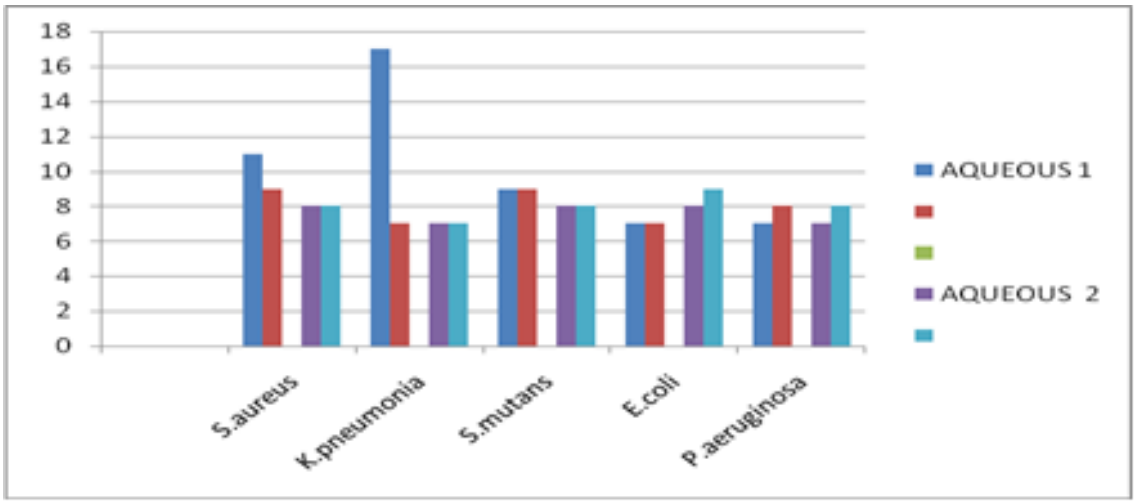

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**Figure 2***IN VITRO* Antibacterial activity of aqueous, methanol and ethanol leaves extracts of *Azadirachta Indica A*

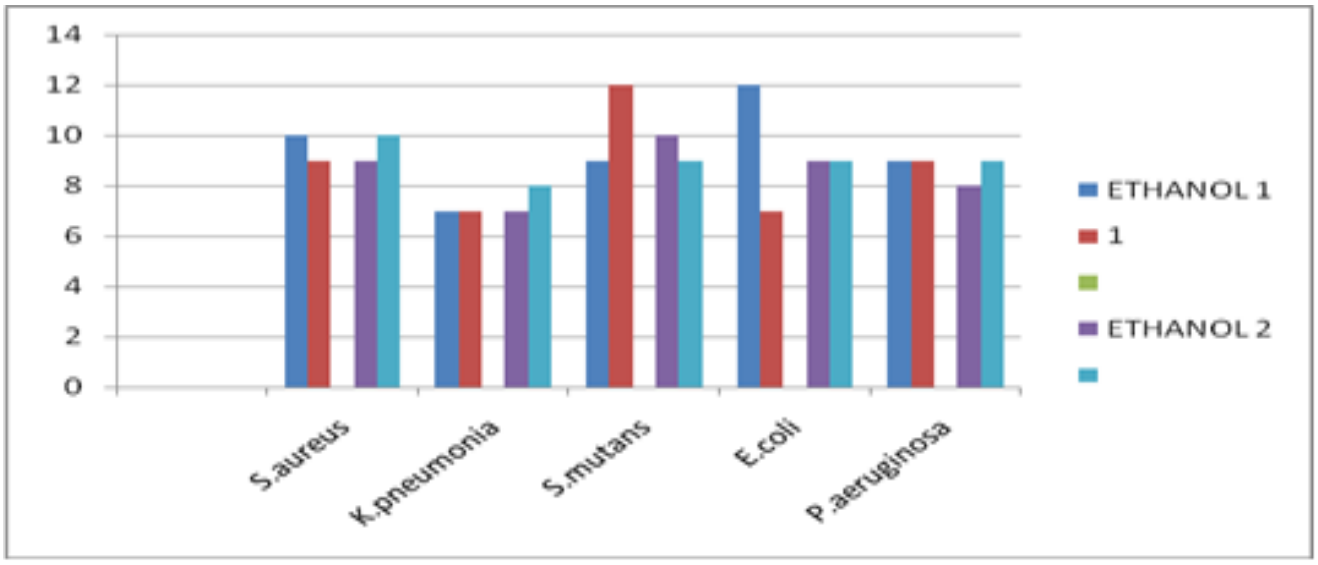


**Figure3:** TLC Separation of aqueous, ethanol, methanol extract of *Solanum Trilobatum*

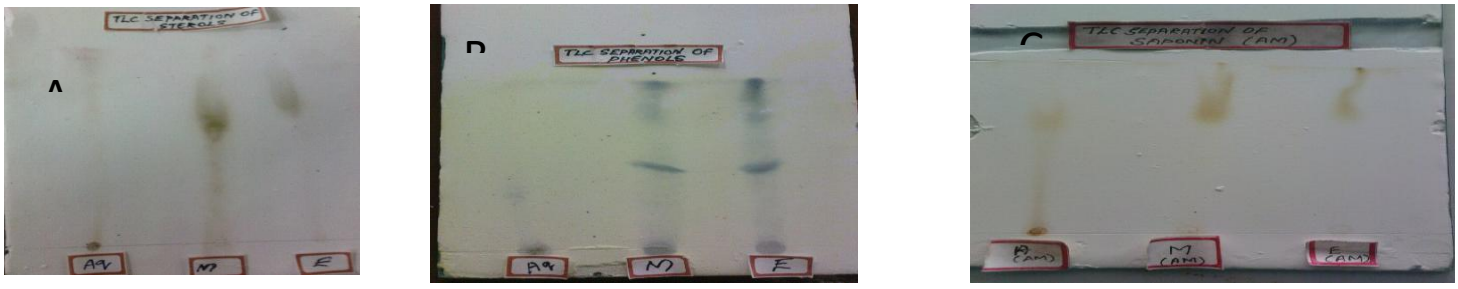


**Figure 6**-TLC Separation of aqueous, ethanol, methanol extract of Azadirachta *Indica A*

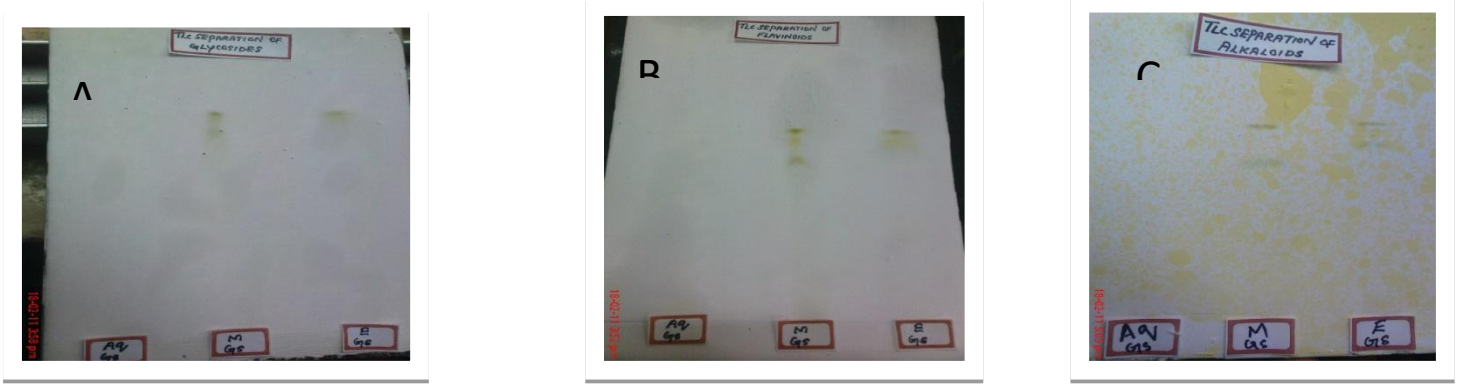

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Figure 5-Antibacterial activity of aqueous,ethanol,methanol extract of *Solanum Trilobatum L*

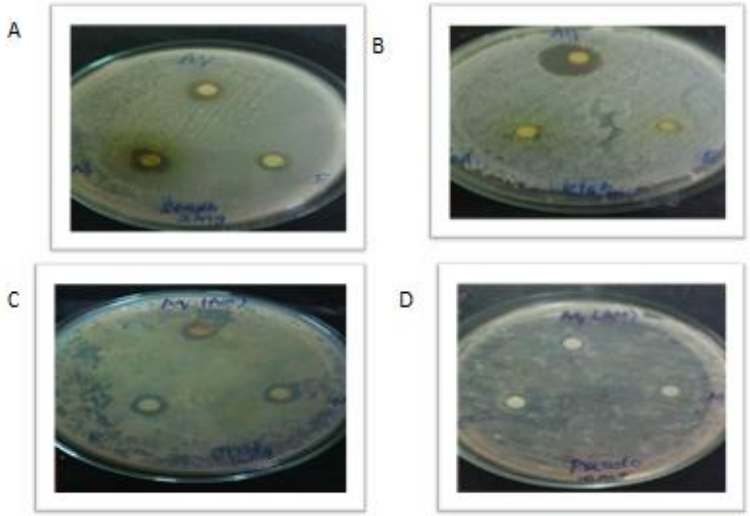


Figure 6 -Antibacterial activity of aqueous,ethanol,methanol extract of Azadirachta *Indica A*

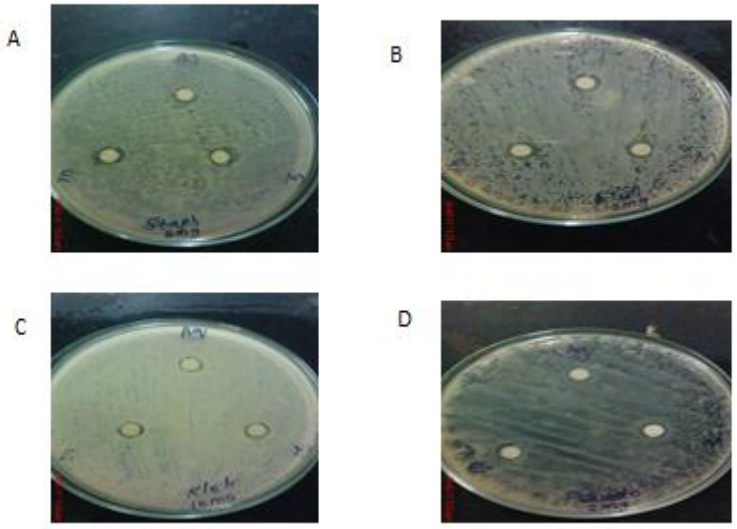

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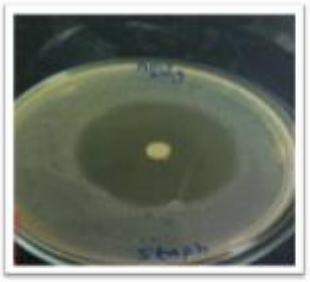
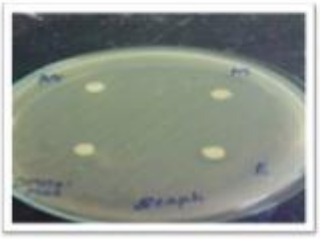
Figure 7-Control activity of Amoxicillin against Staphylococcus aureus

A



Control activity of dimethyl sulphoxide,aqueous,methanol, ethanol

B



The antimicrobial property may be due to their ability to complex with extracellular and insoluble proteins or to complex with bacterial cell walls. Lipophilic flavonoids may also disrupt microbial membrane. It also exhibits anti oxidant activity, reduces the risk of cardiovascular disease. A steroid represents a large group of compounds which exist in nature. It acts as an insulator against nerve impulse which discharges electrical charges (Kirtikar, 2001; Joshi, 2000) Terpenoids are derived from 5 carbon isoprene unit. Terpenoids are active against bacteria, fungi, viruses and protozoa (Pradeepa, 2014). The mechanism of action may involve the disruption of microbial membrane. Cardiac glycosides are secondary metabolites composed of two structural features, sugar (glycosides) and non sugar (aglycone- steroid moieties). It inhibits Na+ - K+ pump and thus it improves cardiac output and reduces distention of heart. It has been reported that carbohydrate and amino acids form complexes with other substances and thus act on the cell membrane of bacteria (Shahzad, 2009; Shokeen, 2009). This showed their importance in exhibiting antibacterial activity.

Separation of phytochemicals by thin layer chromatography was carried out to support antibacterial studies. Aqueous extract of Solanum trilobatum L. was found to have maximum inhibitory activity against klebsiella pneumoniae. S.aureus was found to be moderately inhibited by all the extract. Aqueous, methanol and ethanol extract showed minimum inhibitory activity against E.coli and pseudomonas.

Methanol extract of *Azadirachta* showed maximum inhibitory activity against *Solanum Trilobatum*. All the microorganisms are minimally inhibited by the aqueous methanol and ethanol extracts. The antibacterial activity of the plants may be due to the presence of phytochemical constituents

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## 4. Conclusion

The results of the present study has evidenced that the ethanolic extract of *Solanum trilobatum L. and Azadirachta indica A* leaves contains biologically active secondary metabolites in addition to significant amounts of essential trace elements. The leaves may be considered as an important source for the screening of natural lead molecules. The synergistic effect of both the organic and inorganic compounds present in the *Solanum trilobatum L*. and *Azadirachta indica A* leaves can play a chief role in antibacterial activity and preventing free radical mediated diseases such as diabetes and cancer. Above all, the present study provides a scientific rationale for the use of *Solanum trilobatum L*. and *Azadirachta indica A* leaves in the traditional medicinal system for the treatment of various human ailments. The antibacterial activity may be due to the presence of the active constituents in the plant extracts.

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**CompetingInterests**
Authors have declared that no competing interests exist

**Authors' Contributions**
All the Authors have equal contributions in designing, executing and preparation of manuscript.

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