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Original article

In-vitro antimicrobial activities of *Solanum villosum* (L.) lam; crude extract solvent comparison

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ABSTRACT

Medicinal plants are wealthy resource of antimicrobial agents. In various parts of this world plants are used as medicinal purpose and considered effective resource of medicines. Present study was aimed to investigate the antimicrobial properties of *Solanum villosum*. Flowers, seeds, berries and fresh leaves were collected for aforesaid activity. Agar disc diffusion and agar tube dilution methods were used for anti-bacterial and anti-fungal activities, respectively. Anti-bacterial activity for both extracts (methanol and chloroform) was about 75% against some of the bacterial strains. Anti-fungal activity was observed above 50% for both crude extracts. These positive results are the baseline for next step detailed screening of this plant. Our findings exhibited the worth of *S. villosum*in pharmacology and to support basic healthcare across the globe.

1. Introduction

Plants have been reported previously by showing strong antimicrobial properties (Al-Dhabi et al., 2015). Many studies claimed that the medicinal plants has various phytochemical constituents which mainly pronounced various biological activities such as antibacterial, antifungal, antioxidant and anticancer (Arokiyaraj et al., 2015; Balamurugan, 2015; Barathikannan et al., 2016; Cuong et al., 2017; Elango et al., 2017). Various plant species are used science antiquity against skin diseases for example extract of *Aloe vera*is very caring for dandruff that is a fungal disease and this plant has strong antifungal properties (Antonisamy et al., 2015; Al-Dhabi and Valan Arasu, 2016). Looking ethnobotanical studies a number of plants are screened for antifungal activities and they showed positive results. To cure microbial diseases pharmaceutical companies are paying interest to discover new medicines from plant origin as they are safe to use and less expensive and will be in approach to population (Elango et al., 2016a; Elango et al., 2016b; Fowsiya et al., 2016). Utilization of plants as drug ranges as of 4 to 20% in various countries as well as concerning 2500 species is sold globally (Schippmannet al., 2002; Glorybai et al., 2015).

There is almost 250,000–500,000 plant species reported yet, from these reported species only a small portion has been investigated phytochemically while the percentage to evaluate them biologically and pharmacologically is in fraction (Gurusamy et al., 2019; Haritha et al., 2016; Helan et al., 2016; Ilavenil et al., 2017). In history pharmacological evaluation of compound having natural and synthetic foundations has been the source of numerous curative agents. Arbitrary evaluation as instrument in finding new biologically energetic compounds has been more helpful in the field of antibiotics (Gerhartz et al., 2005).

Medicinal plants are wealthy resource of antimicrobial agents (Kannan and Agastian, 2015; Park et al., 2016a,2016b; Park et al., 2017). In various parts of this world plants are used medicinally and these are effective resource of effective medicines (Park et al.,

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2016a,2016b; Rajkumari et al., 2019; Rathi et al., 2015; Surendra et al., 2016a). A number of medicinal plant parts are used for extract as raw medicine and they course wide-ranging medicinal properties (Surendra et al., 2016b; Valsalam et al., 2019a). Plant parts which are used as raw drugs are roots, stem, flowers, fruits, barks, leaves, latex and other modified plant organs. Local people collect the raw drugs in smaller quantity and locally used to cure diseases. On the other hand many raw drugs are collected in huge amounts and these drugs are traded in market, as these drugs act as the raw material for herbal industries (Uniyal et al., 2006; Surendra et al., 2016c). Plant species are being evaluated for biological activities. Hundreds of them have been investigated for biological activities. However a huge mainstream of them have not been effectively investigated (Liu and Wang, 2008; Valsalam et al., 2019b).

In third world countries, where contagious diseases are common, there should be require to search out and promote those medicines that have origin of plants. These medicines should destroy the microbes causing contagious diseases and should have properties against persistence of contagious diseases. These medicines should stand beside the modern medicines and antibiotics. In this aspect, plants that are being used in conventional herbal remedies will act as great treasure for the investigation and discovery of new drugs having a great extent of antimicrobial properties similar to the modern medicines (Ikram and Hussain, 1978). For the reason that about every antimicrobial agent are being imported and by considering the accessibility of medicinal plants in these countries, a lot of overseas trade can be saved. Remedial flora has been used for centuries like remedies in favor of human diseases as they have mechanism of curative worth (Haq, 1997).

There is an urgent need to investigate new biological active compounds with various chemical structure and unique mode of action. It is noticed that microbes are being resistant to antibiotics. An increase in the incidence of new upcoming contagious diseases is also alarming. Extracts and chemical compounds isolated from plants with known biological and anti-microbial activities can be of great impact in the remedial treatment. Medicinal action of plants is defined to some chemical compounds that impart a specific physiological action on human body. Among these important chemical substances, which are most important and biological active, are flavonoids, alkaloids, tannins and phenolic compounds. Tannins, essential oils and various aromatic compounds are found mostly in leaves; that is the reason, leaves have been found better for anti-microbial principles. These chemicals have been isolated from different plant species and their biological activities have been reported. Plants do not have sufficient ability to synthesize aromatic compounds, phenols and their oxygen substituted derivatives. These chemicals are protective to plants; protect them from microbial infection and weakness (Sheeba, 2010). Keeping in view, current study was designed to investigate the antimicrobial activities of biological active contents of *Solanum villosum* (L.) Lam.

2. Material and methods

2.1. Plant part used

Solanum villosum (L.) Lam. belonging to Solanaceae family was selected for antibacterial and antifungal activity against methanol and chloroform solvent extract. Flowers, seeds, berries and fresh leaves were collected for aforesaid activity.

2.2. Plant material extraction and dose preparation

Plant material collected was washed and rinsed with blotting paper and then placed for shad drying for a week at constant temperature of 18 °Celsius to avoid any useful volatile plant content. Sunlight exposure was highly avoided to restore the volatile and temperature sensitive active content of plants. After shad drying, plant material was grinded to powder.

Methanol and chloroform (80% Analytical Grade) were used as the solvent to extract plant active substances for evaluation of antibacterial and anti-fungal activities. Plant material (100 g) was used for extraction and this was made by soaking of plant material in solvent for about a week a normal room temperature that was avoided from sunlight exposure. During this soaking plant material was stirred ad shake well twice per day. After seven days this extract was filtered via whatman 42 filter paper and extract was collected in a flask. Remaining plant extract was again washed with respective solvent (200 ml) and again filtered and this practice was repeated thrice. Plant extract was concentrated through rotary evaporator by maintain bath temperature at 40 °C. Extracted (Semisolid material) was collected in beaker and placed at room temperature for complete drying. Final extracted plant extract was stored at −4 °C and was used in future for dose preparation in dimethyl sulphoxide (DMSO) for anti-bacterial and antifungal activities (Britto and Senthilkumar, 2005). Preparation of dose dilution is explained in Supporting Information Table 1. Standard antibiotic (doxycycline) was used for comparative studies.

2.3. In-vitro anti-microbial bioassay

Disc diffusion and agar tube dilution methods were followed for the antimicrobial activity studies (Arulvasu et al., 2010; Mahmood et al., 2012b). Anti-bacterial assays were performed against gram positive strains (*Bacillus subtilis* and *Staphylococcus aureus)* and gram negative strains (*Vibrio cholera, Enterobacter aerogenes, Klebsiellapneumoniae, Escherichia coli*. Anti-fungal activity was performed against *Aspergillus nigar* and *Aspergillus* fumigatus.

Disc diffusion method was used for anti-bacterial assays, where agar plats were allowed to solidify and then agar was punched with sterile cork borer. Agar plats were seeded with bacterial strains and a dose of 80 µl test solution was introduced to wells. Positive control was launched by standard antibiotic (doxycycline) and after that plats were incubated for 24 h at 37 °C.

Antifungal activities were screened through agar tube dilution method. Stock test solution was prepared by DMSO solvent. Positive control was launched through terbinafin while DMSO was used as negative control dose. For bioassay 100 µl of test plant material, 100 µl of DMSO and 85 µl of terbinafin was inoculated to agar solution, which was then allowed to solidify. After solidification fungal strains were inoculated to the slants tubes and placed in incubator for 24 h at 37 °C (Fatima et al., 2009).

2.4. Data and statistical analysis

Basic descriptive statistical analysis was performed through Microsoft excel. Antibacterial activities were calculated through measuring the inhibition diameter in millimeters. Antifungal activities were measured by;

$$\text{Inhibition of fungal growth} = 100 - (\text{linear growth in test tube}/\text{linear growth in control}) * 100$$

Data was analyzed by one way ANOVA with 95% level of confidence ($P < 0.05$).

3. Results

3.1. Antibacterial activities

Inhibition zones were recorded in millimeters and dilution doses of crude extracts were prepared in DMSO, as this solvent

Table 1
Results of anti-fungal activities of methanoloic extract of *Solanum villosum.*

Fungi	Solvent	L.G.C. (mm)	L.G.T. (mm)	% Inhibition
A. niger	Methanol	109	63	57.8
A. fumigates		115	89	77.4
A. niger	Chloroform	110	55	50
A. fumigates		113	72	64

has no impact/effect on bacteria. Eight dilutions were prepared and their detail is presented in SI Table 1. 2 mg/ml DOX standard antibiotic was made and 100 μl of each plant extract dilution were introduced into the wells made by sterile cork borer. Results and visual expression for antibacterial activities of methanolic and chloroform plant extract are presented in Figs. 1 and 2. Basic descriptive statistical data is provided in supporting information (SI Tables 2 and 3).

Crude methanolic and chloroform plant extracts were screened against few bacterial strains, which depicted that methnolic crude extract is more potent and effective against tested pathogenic bacterial strains than chloroform extract. Results of one way ANOVA ($P > 0.05$) showed significant different difference between methanolic and chloroform crude extract. Maximum activity of crude extract of methanol was found against *Klebsiella pneumonia* and *Escherichia coli* while least anti-bacterial activity was revealed against *Enterobacter aerogenes* and *Vibrio cholera.* When we screen the crude plant extract in chloroform then dominant activity was found against *Staphylococcus aureus* and *Escherichia coli* while minimum activity was showed against *Vibrio cholera* and *Enterobacter aerogenes.*

In general our results declared that methanol is better solvent for extraction of active constituents as anti-bacterial agents when compared with the chloroform.

3.2. Antifungal activities

Descriptive statistical results for anti-fungal activities are presented in Table 1. Tested strains of fugi against methanol and choloroform were *Aspergillus niger* and *Aspergillus fumigates.* It was revealed from this assay that methnolic extract was more-potant against fungi as compared to chloroform. Maximum anti pathogenic action was observed from methanolic extract against *A. fumigates* (77.4%) followed by the chloroform extract against *A. fumigates* (64%). It was revealed form results that both the tested solvent extracts were more potent against *A. fumigate* strains as compared to the *A. niger*strains.

4. Discussions

Cheapest and safe way to treat the disease is to rely on phytomedicine. Previously, screening of plants originated medicines, their scientific interpretation from traditional and indigenous usage as medicine was started by western scientists (Shakya et al., 2008). Indigenous medicines were subjected to extraction and identification of active substances in nineteen century, followed by formulation and clinical trials (Mahmood et al., 2012b; Hextable, 1993). Presently, peoples across the globe are still relying on plant based medicines/drugs, rather than commercial drugs/product derived or developed from plants (Barrett and Kiefer, 1996).

Biological screening is an expressive technique for the valuable or unhelpful possessions of crude drug or living substance. When medicine is of manifold chemical combinations, then resulting actions are employed by compound's energetic ingredients but can be customized by the other constituents (Souza et al., 2004). The primary type of biological activity is a compound toxicity. Activity is customarily dosage-dependent and is not scarce to express special effects fluctuating as of encouraging to undesirable for one material as available as of low to high doses. While a substance is well thought-out active biologically if it has interference with or upshot on any cell tissue in the human body, biological

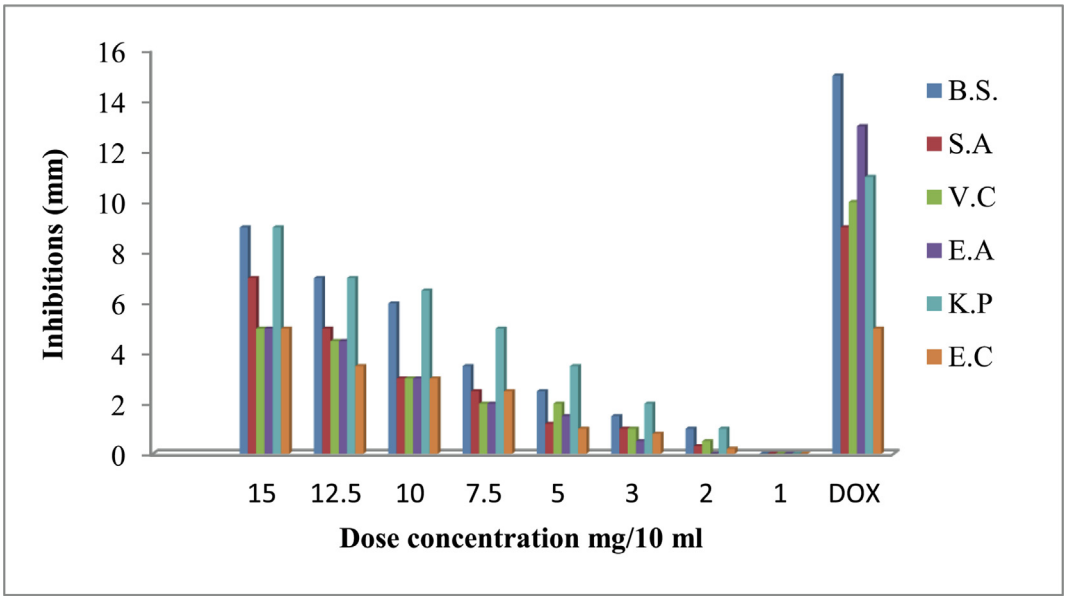

Fig. 1. Inhibitions (mm) against bacterial strains by methanolic plant extract *Bacillus subtilis (B.S), Staphylococcus aureus (S.A), Vibrio cholera (V.C), Enterobacter aerogenes (E.A), Klebsiella pneumonia (K.P), Escherichia coli (E.C).*

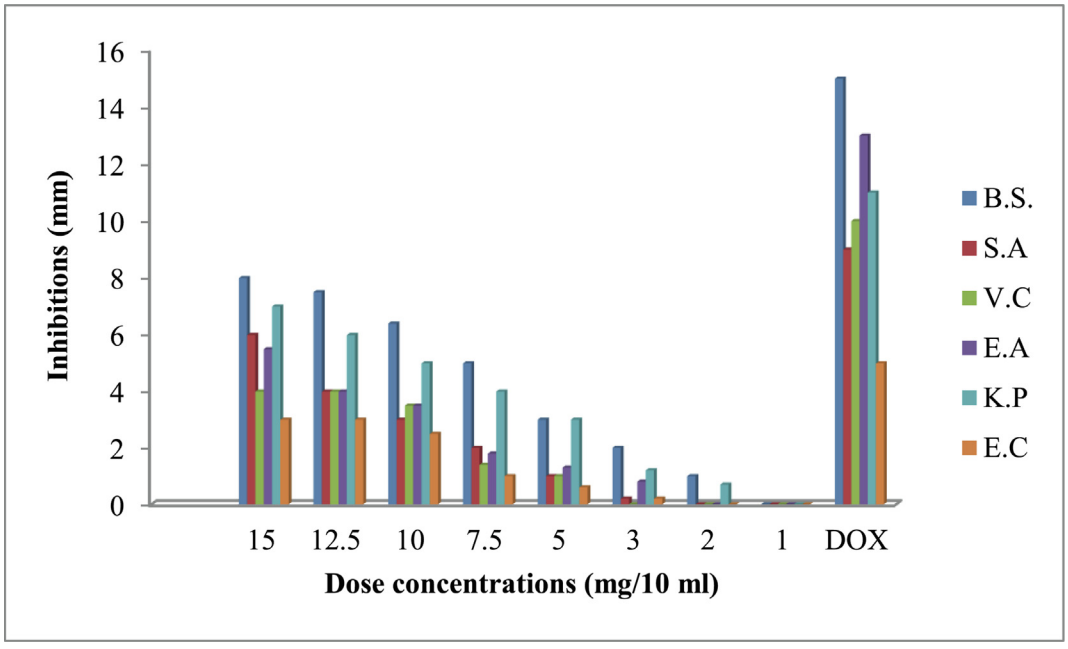

Fig. 2. Inhibitions (mm) against bacterial strains by chloroform plant extract *Bacillus subtilis (B.S), Staphylococcus aureus (S.A), Vibrio cholera (V.C), Enterobacter aerogenes (E.A), Klebsiella pneumonia (K.P), Escherichia coli (E.C).*

activity is typically in use to illustrate positive effects (Vishal et al., 2010).

Solanum villosum (L.) Lam is a perennial medicinal plant used against multiple health problems and belong to the family Solanaceae (Al-Sodany et al., 2013). Current research work presented antimicrobial (anti-fungal and anti-bacterial) activities of methanolic and chloroform crude plant extract of *Solanum villosum.* This attempt was made to explore and verify/validate the reported medicinal uses of aforesaid plant. Antibiotic resistance is a hot core issue in the field of medicines and need special attention to discover new antibiotic drugs based on natural resources with more potent results against pathogens (Anderson and Kaye, 2009; Mahmood et al., 2012a).

*S. villosum*is an operative and active plant against microbial pathogens. Antibacterial and antifungal activities of *S. villosum*categorically evaluated to be dependent on solvent used for crude extraction. Methodology used for both in-vitro activities (Agar well diffusion and agar tube dilution) were more convenient and efficient techniques to investigate the maximum number of samples (extracts) against pathogenic microbes. Methanol was found better solvent, which exhibit maximum antimicrobial activities and provided a new dimension to explore this plant for further detailed pharmacological investigation for real antibiotic drug discovery. On the other hand, our results depicted that all kind of solvent has potential to extract bioactive compounds and considerable activity can be achieved by solvents extraction method. Antibacterial activity for both the extract was about 85% against almost maximum bacterial strains. Anti-fungal activity was observed above 50% for both the crude extracts. These positive results are the baseline for next step detailed screening of this plant.

5. Conclusion

This research paper has exposed that the investigated plant has great potential as antibiotic. *S. villosum*can be considered as good antimicrobial agents. These biological activates of *S. villosum*willhelp to discover new antibiotic that can serve up as selective agents for contagious diseases. Our findings exhibit the worth of *S. villosum*in pharmacology and to support basic healthcare across the globe. These positive results are the baseline for next step detailed screening of this plant.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jksus.2020.01.035.

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