

Phytochemical screening and antibacterial activity of different solvent extracted samples of *Arisaema jacquemontii*

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Abstract: The current research was carried out to assess the antibacterial activities and phytochemical analysis of the methanol, n-hexane, ethyl acetate, n-butanol soluble fractions and aqueous extracts of the tubers of *Arisaema jacquemontii*. All the extracts were tested for their antibacterial potential at 1, 2 and 3 mg disc⁻¹ concentrations against 6 bacterial strains through disc diffusion susceptibility assay. The data suggested that different extracts showed varying degree of growth inhibition against the tested microbes. Statistical analysis revealed that n-hexane and ethyl acetate soluble fractions significantly inhibited the growth of all the bacterial strains at the tested concentrations. Moderate activities were recorded for n-butanol and methanolic extracted samples at different concentrations against all the tested strains of bacteria. *P. aeruginosa*, *S. aureus* and *X. campestris* showed resistance to all the tested concentrations of the aqueous extract. *B. subtilis* and *K. pneumoniae* were resistant at 1 and 2 mg disc⁻¹ concentrations of the aqueous extract and 3 mg disc⁻¹ of the same extract reduced the growth of the same bacteria. Phytochemical analysis of the different solvent extracted samples suggested the presence or absence of various metabolites including alkaloids, saponins, tannins, sterols, flavonoids, protein, carbohydrates and fats.

Keywords: *Arisaema jacquemontii*, antibacterial activity, disc diffusion susceptibility assay, phytochemical analysis.

INTRODUCTION

The use of plants to cure diseases predates written human history. It is due to the ability of plants to synthesize a wide range of biologically active compounds. Being source of many powerful drugs, such medicinal plants and their derived products are known to have antibacterial, and antifungal activities etc (Bakht *et al.*, 2011 a, b, c and d; 2012; 2013 a, b; 2014 a, b, c; Nasir *et al.*, 2015; Amjad *et al.*, 2016; Wajid *et al.*, 2016; Bilal *et al.*, 2017. Herbal medicines do not differ in their mode of action greatly from chemical drugs. This makes plant derived medicines to be as effective as conventional medicines with less allergic responses, side effects and synergy. In this era, the emergence and exponential increase in antibiotic resistance in microbes along with some other problems e.g. allergic reactions have led to the need of screening of plants for new antibacterial drugs (Nisa *et al.*, 2013). Roughly estimated 35,000-75,000 medicinal plants can make a significant contribution as a re-emerging health aid to fulfill the health vacuum (Khalil *et al.*, 2014). About 13,000 plant species worldwide are being used to yield drugs to treat different diseases (Ashfaq *et al.*, 2012). Plants have the ability to synthesize a variety of primary and secondary metabolites. These metabolites are responsible for various important biological functions including antibacterial, antifungal, anti-diabetic, anti-cancer activities etc. These biologically important compounds include glycosides, alkaloids, saponins, resins, oils, tannins, sterols, flavonoids etc. (Ahmad *et al.*, 2013). Phytochemical screening of different plants for

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such bioactive compounds leads to preliminary screening of the plant as potential medicinal plant.

Arisaema jacquemontii, tuberous perennial plant, belongs to the family Araceae, subfamily Aroideae and tribe Arisaemateae. It can be found growing on rocky slopes at an altitude of 2,400-4,000 meters. It is fairly common in the alpine and subalpine forests in East Asia, Afghanistan, Southern India and the Khasi Hills region in north-east India and Himalayas. It dies back to ground level in the winter or dry season. The oxalic acid and calcium oxalate crystals (raphids) are present in all parts of the plant. It can only be eaten or used after being properly processed, fermented or cooked. The tubers are crushed and its juice is used for treating ringworm and various other skin diseases in India. A lectin has been isolated from tuber and purified which contain insecticidal and anti-proliferative activity. Different parts of the plant and its preparations are used to cure different ailments in folk medicine system (Tanveer *et al.*, 2013).

MATERIALS AND METHODS

Collection of plant material

The plant material (tubers) of *Arisaema jacquemontii* (locally known as marjarrai) was collected from the mountainous areas of Kalam (Swat), Khyber Pakhtunkhwa province of Pakistan. The tubers were thoroughly washed with tap water followed by distilled water to remove soil particles and other debris. The plant sample was shade dried at room temperature until dried and grinded to fine powder using an electric grinder.

Preparation of crude extract

The grinded tubers were infused in 1.5liters analytical grade methanol for 7 days with intermittent shaking to dissolve the bioactive compounds. After 7 days, the sample was filtered through Whatman filter paper No. 1. The remaining residues were again soaked in fresh methanol for 1 day, filtered and the same process was repeated for the third time. The obtained methanolic filtrate was dried using rotary evaporator under reduced pressure at 45°C. This dried methanolic extract was divided into two parts; one to be used as crude methanolic extract and other to be fractionated with n-hexane, ethyl acetate and n-butanol.

Fractionation of methanolic extract

The crude methanolic extract to be fractionated was suspended in 300ml distilled water and partitioned with 300ml of n-hexane using separating funnel. The funnel was shaken well and allowed to stand for 15 minutes until two separate layers were formed, the upper being n-hexane layer and lower being aqueous layer. The n-hexane layer was collected and this process was repeated three times by adding fresh n-hexane to aqueous phase. All n-hexane fractions were combined together and dried under reduced pressure using rotary evaporator at 45°C. The same procedure was followed for ethyl acetate and n-butanol. Aqueous extract obtained at the last stage was dried as described earlier.

Disc diffusion susceptibility assay for antibacterial activity

For culturing and growth of bacteria to carry out disc diffusion assay, nutrient agar media (HiMedia Laboratories Pvt. Ltd.) was used. Nutrient broth was used for shaking, incubation and standardization of different microorganisms. Growth media was prepared as described by Bakht *et al.* (2011 a). Antibacterial activity of the extracts was evaluated by the methods of Bauer *et al.* (1966). Stock solutions of the different extracts were prepared in sterile DMSO. The plates were inoculated using microbial inoculums under sterile conditions. Three discs of Whatmann No. 1 filter paper (6mm diameter) were placed on the petri plates using sterilized forceps. Three volumes i.e., 6, 12 and 18µl from each stock solution were applied on discs respectively corresponding to three concentrations of the extracts i.e. 1, 2 and 3mg disc⁻¹. The plates were labeled, properly sealed by parafilm and incubated at 37°C for 24 hours. Antibiotics were used as positive control while DMSO as negative control. After incubation, zone of inhibition was measured in millimeters in comparison with positive control. The same procedure was repeated for each micro-organism. The percent (%) inhibition was finally calculated by the following formula.

$$\% \text{ inhibition} = \frac{\text{Zone of inhibition of sample (mm)}}{\text{Zone of inhibition of control (mm)}} \times 100$$

Positive controls

For Gram-positive bacteria; Ciprofloxacin 50 µg per 6µl
For Gram negative-bacteria; Ciprofloxacin 50 µg per 6µl

Negative control

DMSO was used as negative control.

Microorganisms tested

Antibacterial activity of different solvent extracted samples from tubers was tested against different bacterial and fungal strains (table 1).

STATISTICAL ANALYSIS

The experiment was repeated in triplicate and MSTAT computer software was used for the analysis of the data. Least Significant Difference (LSD) test was employed upon obtaining significant difference at p<0.05 (Steel *et al.*, 1997).

RESULTS

Different solvent extracted samples from the tubers of *Arisaema jacquemontii* showed various levels of antibacterial activity against different tested bacterial strains assessed by disc diffusion method (fig. 1). The data indicated that highest activity against *B. subtilis* was shown by the ethyl-acetate fraction measuring 40.8% zone of inhibition (ZI) at 3mg disc⁻¹ concentration followed by 35.6% and 30.9% ZI at 2 and 1mg disc⁻¹ respectively of the same extract compared with controls. The data also showed 38.2% inhibitory activity by n-hexane extracted samples at concentration of 3mg disc⁻¹. Moderate activity of 33.9% was recorded by methanolic extract at 3 mg disc⁻¹, 32.1% by n-hexane extract at 2mg disc⁻¹ and 30.9% by n-butanol extract at 3mg disc⁻¹

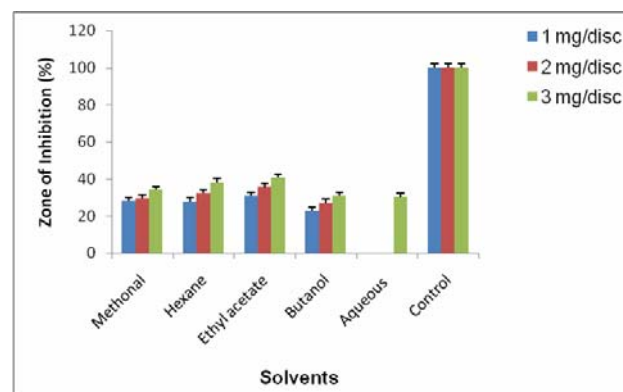


Fig. 1: Antibacterial activity of crude methanol, n-hexane, ethyl acetate, n-butanol and water extracted samples from fresh samples of *Arisaema jacquemontii* against *B. subtilis* by disc diffusion assay (Bar shows LSD at p<0.05).

concentration respectively. The aqueous extracts did not show any antibacterial activity against *B. subtilis* at low concentration of 1 and 2mg disc⁻¹. However, 30.3% inhibitory activity was recorded for aqueous extract at higher concentration of 3mg disc⁻¹.

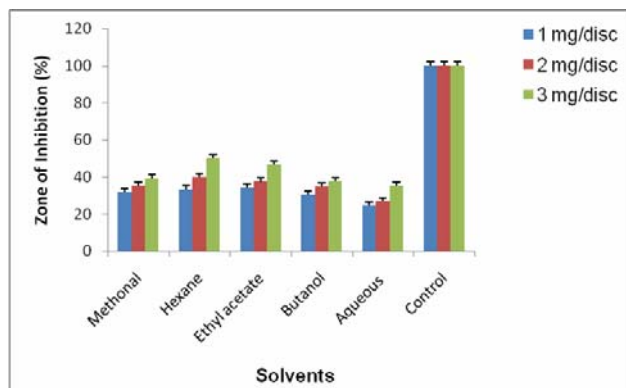


Fig. 2: Antibacterial activity of crude methanol, n-hexane, ethyl acetate, n-butanol and water extracted samples from fresh samples of *Arisaema jacquemontii* against *E. coli* by disc diffusion assay (Bar shows LSD at $p < 0.05$).

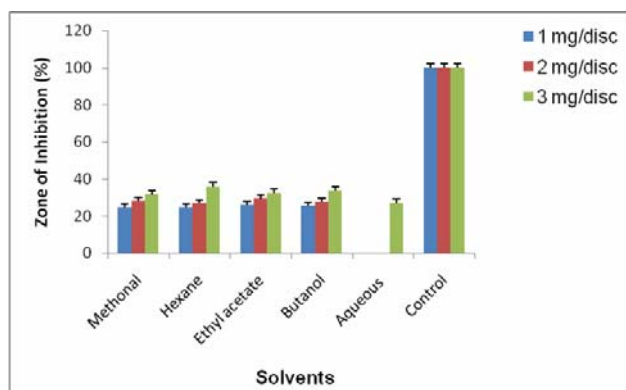


Fig. 3: Antibacterial activity of crude methanol, n-hexane, ethyl acetate, butanol and water extracted samples from fresh samples of *Arisaema jacquemontii* against *K. pneumonia* by disc diffusion assay (Bar shows LSD at $p < 0.05$).

The antibacterial activity of different solvent extracted samples against *E. coli* is shown in fig. 2. It is evident from the data that the tested bacterium showed highest susceptibility to n-hexane soluble fraction measuring 50% inhibitory activity at 3mg disc⁻¹ concentration followed by 39.68% and 33.2% activity at 2 and 1mg disc⁻¹ concentration respectively of the same extract. The data also indicated that 46.4% ZI was recorded for ethyl-acetate extracted fraction at concentration of 3mg disc⁻¹. Moderate activities of 39% by methanolic extract at 3mg disc⁻¹, 37.4% by ethyl-acetate extract at 2mg disc⁻¹, 37.5% by n-butanol extract at 3mg disc⁻¹ concentration and 35.4% by aqueous fraction at 3mg disc⁻¹ was recorded compared with controls. Lowest activity against *E. coli*

was shown by aqueous fraction at 1 and 2 mg disc⁻¹ recording 26.7% and 24.5% inhibitory zone respectively.

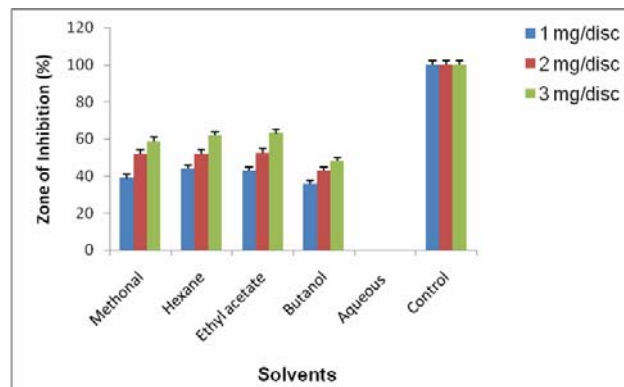


Fig. 4: Antibacterial activity of crude methanol, n-hexane, ethyl acetate, n-butanol and water extracted samples from fresh samples of *Arisaema jacquemontii* against *P. aeruginosa* by disc diffusion assay (Bar shows LSD at $p < 0.05$).

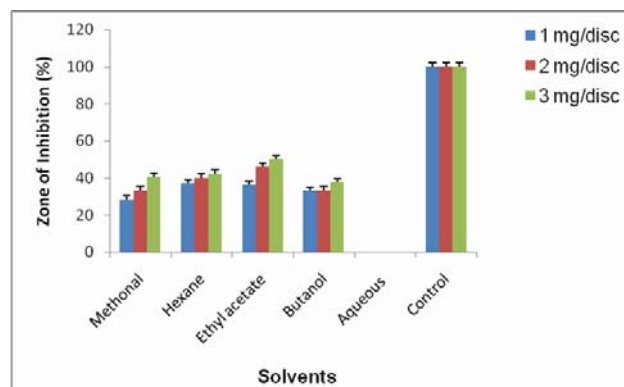


Fig. 5: Antibacterial activity of crude methanol, n-hexane, ethyl acetate, n-butanol and water extracted samples from fresh samples of *Arisaema jacquemontii* against *S. aureus* by disc diffusion assay (Bar shows LSD at $p < 0.05$).

Fig. 3 shows the antibacterial activities of different solvent soluble fractions of tubers against *Klebsiella pneumonia*. The data indicated the highest growth inhibition of 35.9% against the bacteria by n-hexane extracted sample when applied at 3mg disc⁻¹ concentration followed by the 26.7% and 24.5% ZI at 2 and 1mg disc⁻¹ respectively of the same extract in comparison with controls. The data also showed 33.7% inhibitory activity by n-butanol soluble fraction at concentration of 3 mg disc⁻¹. 31.6%, 25.8%, and 29.3% inhibitory activity was shown as moderate activity by methanolic extract at 3 mg disc⁻¹ and ethyl-acetate extract at 1 and 2 mg disc⁻¹ concentration respectively. Lowest activity against the pathogen was recorded as zero at concentration 1 and 2 mg disc⁻¹ of aqueous extract. The same aqueous extract showed some inhibitory activity of 27% at concentration of 3 mg disc⁻¹.

Table 1: Microbial strains used during the experiment

Microbial Species	Gram strain type	Details of the Microbial strains used
<i>K. pneumoniae</i>	Negative	Clinical isolate obtained from The Department of Microbiology, Microbiology, Quaid-I-Azam University Islamabad, Pakistan
<i>P. aeruginosa</i>	Negative	ATCC # 9721
<i>S. aureus</i>	Positive	ATCC # 6538
<i>B. subtilis</i>	Positive	Clinical isolate obtained from The Department of Microbiology, Microbiology, Quaid-I-Azam University Islamabad, Pakistan
<i>E. coli</i>	Negative	ATCC # 25922
<i>X. campestris</i>	Negative	ATCC # 33913

Table 2: Phytochemical screening of different solvent extracted fractions of *Arisaema Jacquemontii*

Plant Extract	Alkaloid	Saponin	Tannin	Sterols	Flavonoid	Protein	Carbohy-drates	Oils
Methanol	++	+	+++	+++	+++	+++	+++	+++
n-Hexane	—	—	—	+++	+++	—	+	+++
Ethyl acetate	—	+++	++	++	+++	+	+	—
n-Butanol	+	+++	+++	++	+++	++	+++	++
Aqueous	+	++	+++	++	++	+++	+	+++

+++ : shows the presence in abundance
 ++ : shows presence in moderate quantity
 + : shows presence but in less amount
 — : shows complete absence of the compound.

The data shown in fig. 4 refers to the antibacterial activity against *P. aeruginosa*. The data showed that maximum ZI of 62.9% was noted against *P. aeruginosa* by ethyl-acetate fraction at 3mg disc⁻¹ followed by 52.5% and 42.9% ZI at 2 and 1mg disc⁻¹ of the same fraction. Furthermore, 61.7% inhibitory activity was showed by n-hexane fraction at 3mg disc⁻¹. Moderate activity of 58.7%, 52.08% and 52% was recorded for 3 and 2mg disc⁻¹ concentration of methanolic extract and 2mg disc⁻¹ concentration of n-hexane fraction respectively. Low inhibitory activity of 35.8% was recorded by n-butanol fraction at 1mg disc⁻¹ concentration. The data further revealed that no activity was shown by aqueous extracted fraction against *P. aeruginosa* at all the 3 tested concentrations measuring 0% ZI.

Highest growth reduction of 50% was revealed by ethyl-acetate soluble fraction against *S. aureus* at 3mg disc⁻¹ followed by 46% and 36.2% at 2 and 1mg disc⁻¹ concentrations respectively of the same sample compared with controls (fig. 5). It is also evident from the data that 42.2% growth inhibition was shown by 3mg disc⁻¹ of n-hexane sample. Moderate growth inhibition of 40.5%, 40%, 37.7% and 37.2% was measured at 3mg disc⁻¹ of methanolic crude extract, 2mg disc⁻¹ of n-hexane, 3mg disc⁻¹ of n-butanol and 1mg disc⁻¹ of n-hexane extracted samples respectively. Relatively lesser inhibitory activity (37.7% ZI) was reported at 3 mg disc⁻¹ concentration of n-butanol extract. While 33.3% ZI was recorded for 2 mg disc⁻¹ of crude methanolic and n-butanol extract. *S. aureus* was found to be resistant to all the tested concentrations of the aqueous extracted samples showing no zone of inhibition.

The antibacterial activity of different solvent soluble fractions against *X. campestris* is shown in Figure 6. Analysis of the data indicated that maximum reduction in the growth of the tested bacterium (53.3% ZI) was observed at 3mg disc⁻¹ concentration of n-hexane followed by 41.5% and 35% inhibitory activity at 2 and 1 mg disc⁻¹ concentration of the same fraction. The data also showed that 50.5% ZI was measured at 3 mg disc⁻¹ concentration of ethyl-acetate extracted samples. Moderate activity of 45.2%, 42.9% and 40.9% were observed at 2mg disc⁻¹ of ethyl acetate, 3 mg disc⁻¹ each of n-butanol and crude methanolic extract respectively. Minimum growth inhibition of 39.2% ZI at 2 mg disc⁻¹ of n-butanol, 37.9% ZI at 2 mg disc⁻¹ of crude, 35.3% ZI at 1 mg disc⁻¹ of n-butanol and 33.2% ZI at 1 mg disc⁻¹ of ethyl acetate extracted samples. It is also clear from the data that no activity was shown by aqueous extracted fraction against *X. campestris* at all the tested concentrations.

Phytochemical screening of the different extracts from *Arisaema jacquemontii* revealed that methanolic crude extract contain tannins, sterols, flavonoids, protein, carbohydrates, oils in higher, alkaloid in moderate while saponins in lesser concentration. The n-hexane soluble samples possessed sterols, flavonoids and oils abundantly and carbohydrates less abundantly. Ethyl acetate fraction showed the presence of flavonoids and saponins in higher quantity, tannins and sterols in moderate while protein and carbohydrates in lesser amounts. The data further suggested that saponins, tannins, flavonoids and carbohydrates in higher, sterols, proteins and oils in moderate and alkaloids in lesser amount were present in n-butanol fraction of the tubers. Moreover, high

concentration of tannins, proteins and oils, moderate concentration of saponins, sterols and flavonoids and lesser amount of alkaloids and carbohydrates were found to be present in aqueous extract of *Arisaema jacquemontii* (table 2).

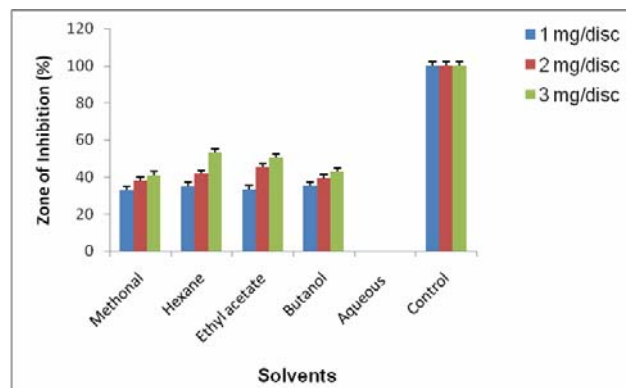


Fig. 6: Antibacterial activity of crude methanol, n-hexane, ethyl acetate, n-butanol and water extracted samples from fresh samples of *Arisaema jacquemontii X. campestris* by disc diffusion assay (Bar shows LSD at $p < 0.05$).

DISCUSSION

Analysis of the data showed that maximum reduction in growth of *B. subtilis* was noted by ethyl acetate fraction at the highest concentrations compared with other samples and controls. N-hexane fraction was also effective to control the growth of the same microbe at 3mg disc⁻¹ concentration. In comparison with other samples and controls, moderate activity was recorded for crude methanolic extract and n-butanol extracted samples at all the tested concentrations. No inhibitory activity was exhibited by aqueous extracted samples at concentrations of 1 and 2mg disc⁻¹. The same extract at higher concentration of 3mg disc⁻¹ showed some inhibitory activity against the tested microbe. The results are in agreement with Bibi *et al.* (2011) who investigated the antibacterial potential of some selected plants of Pakistan and found that ethyl acetate fraction of the rhizome of *Arisaema flavum* actively inhibited the growth of *B. subtilis*. It is evident from the data that *E. coli* was susceptible to all of the tested extracts and revealed growth inhibition activities against the tested microbe. N-hexane soluble fraction was found to be highly effective in inhibiting the growth of *E. coli* at the 3 mg disc⁻¹ concentration compared with other samples and controls. The data also indicated that ethyl acetate extracted sample was active at its highest concentration. It was found that methanolic, n-butanol and aqueous extracted samples at highest concentration showed moderate activity. Lowest activity against the same microbe was recorded for the aqueous extracted samples applied at 2 and 1mg disc⁻¹ concentrations. These findings are in consensus with Baba and Malik (2015). The researchers recorded MIC values for methanolic extract of roots of *Arisaema jacquemontii*

against some Gram-positive and Gram-negative bacteria and suggested that the methanol extract significantly inhibited the growth of *E. coli*, *B. subtilis*, *S. aureus* and some other tested microbes.

Analysis of the data collected for the antibacterial activity of different solvent extracted samples against *K. pneumonia* revealed that n-hexane soluble fraction was the most active (at 3mg disc⁻¹ concentration) of all the tested samples against *K. pneumonia* compared with the other samples and controls. N-butanol fraction was also effective against the microbe at highest concentration of 3 mg disc⁻¹. Crude methanolic and ethyl acetate extracted samples measured moderate activity at all the tested concentrations. Aqueous extracted fraction showed no activity at the concentrations of 2 and 1mg disc⁻¹ and less activity at 3mg disc⁻¹ concentration. Siswati *et al.* (2013) reported growth inhibitory activities of stem, roots and leaves of some plants of Araceae against many microbes including *E. coli*, *K. pneumoniae*, *S. aureus*, *B. subtilis* and *Pseudomonas* species. Our results also show consensus with Dhanraj *et al.* (2013) who reported the effectiveness of the leaf extract of *Colocasis esculenta* against *K. pneumonia*, *P. aeruginosa*, *B. subtilis* and *E. coli*. *P. aeruginosa* showed maximum susceptibility to ethyl acetate extract at 3 mg disc⁻¹ concentration compared with controls and other samples. Ethyl acetate extracted fraction was also active at all the tested concentrations against the tested microbe. Moderate activity was shown by crude methanolic extract and relatively lower activity was recorded for all the three concentrations of the n-butanol extracted samples. The results are similar to the findings of Mohan *et al.* (2008). They reported profound antibacterial activity of tubers of *Typhonium flagelliforme* against some Gram positive and Gram negative bacteria including *P. aeruginosa*. Roy *et al.* (2012) reported active growth inhibition of *S. aureus*, *E. coli* and *P. aeruginosa* by crude methanolic, ethyl acetate and chloroform extracted fractions of the aerial parts of *Typhonium trilobatum* Linn.

The antibacterial activity of the samples and controls against *S. aureus* suggested the highest reduction in growth at 3 mg disc⁻¹ concentration of ethyl acetate extracted fraction. The data also indicated that the highest concentration of the n-hexane extracted fraction was also effective to reduce the growth of the same microbe. Moderate activity was recorded for the crude methanolic and n-butanol extracted samples at all the tested concentrations. Syed *et al.* (2014) observed the highest antibacterial activity of n-hexane and ethyl acetate and crude extracts of *A. calamus* against *S. aureus*. N-hexane fraction at 3 mg disc⁻¹ concentration was very effective to control the growth of *X. campestris* when compared with controls and others samples. Ethyl acetate extracted fraction at highest concentrations inhibited the growth of the tested microbe. Upon comparison with controls and

other samples, moderate activity was found to be carried out by all the tested concentrations of crude methanolic and n-butanol extracts. Similarly, *X. campestris* also showed susceptibility to the tested plant extracts of the tested plant species (Britto *et al.*, 2011). Moreover, analysis of the data also suggested that *P. aeruginosa*, *S. aureus* and *X. campestris* showed complete resistance to all the three tested concentrations of the aqueous extracted samples. Phytochemical screening of different solvent extracted samples showed the presence of several bioactive compounds in varying quantities including tannins, sterols, flavonoids, saponins, alkaloid, protein, carbohydrates and oils. The presence of these bio-active compounds may be responsible for their antibacterial activity.

CONCLUSION

From these results it can be concluded that different solvent extracted from *Arisaema jacquemontii* reduced the growth of different microbial strains under study. Among the solvent, n-hexane and ethyl acetate extracted fractions were more effective to inhibit the activity of the tested microbes. Phytochemical screening revealed the presence of alkaloids, saponins, tannins, sterols, flavonoids, protein, carbohydrates and fats.

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