Bulletin of Pure and Applied Sciences.
Vol.38 B (Botany), No.1. Jan-Jun 2019: P.23-33

Original Article

Print version ISSN 0970 4612
Online version ISSN 2320 3196
DOI: 10.5958/2320-3196.2019.00004.1
Available online at www.bpasjournals.com

Antibacterial and Antioxidant Potential of *Arisaema jacquemontii* Blume from Manali, Himachal Pradesh

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Received on 21.01.2019,
Accepted on 04.03.2019

Keywords:
Arisaema jacquemonti Blume,
Antibacterial activity,
Agar-well diffusion method,
Percent inhibition of bacterial growth,
Antioxidant potential,
DPPH free radical scavenging assay.

Abstract

Manali is not only a famous tourist place but also a treasure of many traditional medicines. The antibacterial and antioxidant potential of leaves, tubers and fruit of *Arisaema jacquemonti* Blume were evaluated in different extracts *viz.* acetone, chloroform, distilled water and methanol. The samples were collected from the area of Manali. Using Agar-well diffusion method and different concentrations of extracts (25%, 50%, 75% and 100%), the antibacterial activity was tested against three Gram-negative bacteria (*Salmonella typhi, Pseudomonas aeruginosa* and *Shigella dysenteriae*) and two Gram-positive bacteria (*Lysteria monocytogenes* and *Staphylococcus aureus*). Streptomycin (5 µg/ml) was taken as a standard antibiotic. Percent inhibition of bacterial growth was calculated for each tested extract. The tuber acetone, fruit methanol and leaf methanol extract were found to be more effective against some the tested bacteria than the standard antibiotic used. The chloroform and distilled water extract showed negligible results. IC_{50} value was calculated after 2, 2 Diphenyl-1-picrylhydrazyal (DPPH) free radical scavenging assay to investigate the antioxidant potential at different concentrations (20, 40, 60, 80 and 100 µg/ml). Ascorbic acid at similar concentrations was taken as standard (IC_{50} = 31.33µg/ml). The methanol-leaf extract was found to exhibit highest antioxidant potential with IC_{50} value of 47.89µg/ml followed by methanol-tuber, water- fruit, acetone-fruit and methanol-fruit, acetone-tuber, acetone-leaf and water-leaf extracts. The results can be taken as a base for further analysis and isolation of active compounds of this plant.

INTRODUCTION

Infectious diseases affect about 50,000 people every day[1]. *Staphylococcus aureus* is typical bacterium causing number of skin disorders. Food borne illnesses are one of the major problems in the world which are mostly contributed by bacteria like *Salmonella* spp. and *Listeria monocytogenes*. *Pseudomonas aerugenosa* is one of the top three contributors of opportunistic human infections[2]. *Shigella dysenteriae* is

associated with the poor sanitation and overcrowded areas of the world. Beside these issues, the imprudent use of commercial and synthetic antimicrobial drugs for the treatment of infections has developed multiple drug resistance in pathogenic organisms[3,4]. In 2003, Finch[5] reported *S. typhi* attaining MDR to the first line of antibiotics. Synthetic antibiotics are also associated with allergy, hypersensitivity and immune suppression[6].

With changing life style and environmental conditions, several chemical molecules such as free radicals are exposed which are not appropriate for human health. Being extremely unstable, these radicals draw electrons out from other molecules by deteriorating them to attain stability. Oxygen is vitally important but its toxicity to living tissues can't be denied. Number of Reactive Oxygen Species (ROS) such as hydroxyl radicals, superoxide anion radicals and hydrogen peroxide radicals are the crucial part of metabolism. In adequate concentration they are essential for energy supply, detoxification, chemical signalling and immune responses[7]. But increased concentration may lead to the consequences like oxidative stress relating to the initial symptoms of variety of suffering including cancer, inflammations, age related diseases and Neuro-degradation[8-12]. Plants have tremendous potential in the field of medicines because they are effective against infections and also, they mollify the side effects commonly associated with the synthetic drugs[13]. Plants produce large number of secondary metabolites such as phenols, flavonoids, anthocyanins, carotenoids, dietary glutathionine and vitamins which are the main components of defence system[14]. These molecules present in the herbal remedies have the ability to combine with many other inactive substances and thus provides a plant much efficiency and superiority to its isolated and pure active compounds[15]. These metabolites also act as antioxidants and function as singlet or triplet oxygen quenchers, peroxide decomposers, enzyme inhibitors and synergists[16]. Between 1981 and 2002, about 61% of the developed drugs in the field of infectious disease and cancer were based on natural products[17]. Even today, 80% of the world population is dependent on traditional remedies for the treatment of various diseases. Traditionally, specific plants are being used as medicine for specific diseases[18].

Arisaema jacquemontii Blume of Araceae family is native to sub-alpine Himalayan region, distributed at the heights between 2,500-4,000 m in Afganistan, Pakistan, India (Jammu and Kashmir, Himachal Pradesh, Uttrakhand, Sikkim and Arunachal Pradesh), Nepal, Tibet and Bhutan[19]. In Himachal Pradesh, this plant is reported from Kinnaur, Kullu, Mandi and Shimla districts, preferring the cervices between stones under the shade of oak and deodar trees on loamy, peaty and well drained but moist soil full of humus. In Kulluvi dialect, it is known as *Basair*. In traditional medicine of local area, tubers of this plant are being used for dermatological infections and ringworms[20]. This plant is also used for boils, blisters, psychic and nervous disorders in other areas where this plant grows[21-23]. Whole plant is also being used against snake bites, microbial infections, swelling, throat infection, obstructions, infertility and uterus diseases[24-25]. In Tibetan therapy, the flower, tuber and tuber is used against scabies, toothache, swellings, chest infection, menstrual disorders and throat problems[26]. Keeping this ethno medical knowledge and above mentioned challenges in mind, the present investigation was carried out to find out the antibacterial and antioxidant potential of the plant in different solvents using different concentrations.

MATERIALS AND METHODS

Collection of plant material

The study material was collected from the Solang Valley at an altitude of 2353–2543m in Manali, Kullu district, Himachal Pradesh. The leaves were collected at the end of July 2018 and tuber and fruit were collected at the first week of September. The plant was identified at Department of Biosciences, HPU. Collected leaves, fruit and tuber were washed properly under tap water to remove dirt and soil particles and were surface sterilized using 0.1% of mercuric chloride solution. This material was dried and ground coarsely in mortar- pestle for further use. The experiments were conducted in the month of October and November.

Preparation of plant extracts

Acetone, chloroform, methanol and water extracts of dried fruit, tubers and leaves were prepared to screen antimicrobial activity. 3 g of material was taken in Erlenmeyer flask to which 30 mL of required solvent was added. The flask was covered with aluminium foil and placed at safe place for 3-5 days for extraction. Material was filtered using Whatman filter paper no.1 and the extract was evaporated at 40°C using rotary evaporator. The extract was collected and weighed. At last, a stock solution of 50 mg/mL concentration was prepared.

Procurement of bacteria

Different human Pathogenic bacteria (*Listeria monocytogenes, Pseudomonas aureginosa, Salmonella typhi, Shigella dysenteriae* and *Staphylococcus aureus,*) have been procured from IGMC, Shimla and Department of Biotechnology, HPU, Shimla for screening antibacterial properties of different plant extracts. The collected pathogens were revived fortnightly in nutrient broth and were stored at 4°C in refrigerator. Pure cultures of all the bacteria were maintained on nutrient agar medium slants and preserved in refrigerator at 4°C. Sub-culturing was done at regular intervals in order to maintain the cultures.

Screening of prepared extracts for antibacterial activity

Different extracts (methanol, acetone, chloroform and water) of plant were screened using Agar-well diffusion method. Nutrient agar medium (Beef extract 1 g, Yeast extract 2 g, Sodium Chloride 1 g, Peptone 5 g, Agar 20 g, Distilled Water 1,000 mL) was prepared and autoclaved at 121.6^{o}C for 30 minutes. 20 mL of hot, liquid nutrient agar medium was poured into each of the Petri plates and let it to set for 20 minutes. Bacteria were grown in nutrient broth for 24 hours prior to screening experiment. Bacteria were spread on solid nutrient agar plates with the help of Spreader. Five wells of 8 mm diameter were bored with the help of sterilized stainless steel cork borer in each Petri plate. Then the wells in each plate were loaded with 25%, 50%, 75% and 100% concentration of prepared plant extracts. The well kept as a control contained pure solvent only. The plates were incubated at 37±2^{o}C for 24 hours in the incubation chamber. The zone of growth inhibition was calculated by measuring the diameter of the inhibition zone around the well (in mm) including the well diameter. The readings were taken in perpendicular direction in all the three replicates and the average values were noted. Percentage inhibition of bacterial species was calculated after subtracting control from the values of inhibition zone diameter obtained and using positive control i.e. Streptomycin, as standard.

$$\%\text{growth inhibition} = \frac{(\text{test} - \text{control})}{\text{standard}} \times 100$$

DPPH Radical Scavenging Activity Assay

The antioxidant potential of the plant was determined by using 2, 2 Diphenyl-1-picrylhydrazyal (DPPH) assay as described by Blois[27] in 1958 with some modifications. From the stock of 100 µg/mL, different concentrations were prepared by serial dilution method. To 1 mL of the extract (i.e. 20, 40, 60, 80 and 100 µg/mL) 1ml of DPPH (0.1 mM in methanol) was added. Corresponding blank samples (acetone, methanol and water) were prepared in which only 1 mL of solvent and 1mL of DPPH solution was added. Ascorbic acid in the same concentrations was used as standard reference. The prepared set for antioxidant potential screening was allowed to stand for 30 minutes in dark. Absorbance was measured by UV-VIS spectrophotometer, at λ 517 nm. All tests were carried out six times each. The percentage of inhibition was calculated by using the formula:

$$\%\ \text{DPPH Scavenging} = \frac{\text{A control} - \text{A sample}}{\text{A control}} \times 100$$

Where, $A_{control}$ is the absorbance of the control and A_{sample} is the absorbance of the sample[27].

Graphs were plotted against % DPPH scavenging v/s concentration of plant extract and standard ascorbic acid in order to find out the values of the slope and y- intercept. IC-50 value (the amount of antioxidant required to decrease the initial DPPH conc. by 50%) for each extract and ascorbic acid was evaluated by using the following equation[28]:

IC_{50} value = $\frac{50 - \text{Y intersept}}{\text{slope}}$

Statistical Analysis

Data are presented as mean± standard error of multiple time repeated experiments. To find out the IC_{50} value Regression value and value of Y-intercept were calculated using Microsoft Office Excel 2007.

RESULTS

Screening of prepared extracts for antibacterial activity

Streptomycin is a broad spectrum antibiotic which displayed great inhibition zone against all the tested bacteria. Chloroform and Distilled water extracts did not inhibit the growth of any of the tested bacteria.

A. Acetone extracts

Acetone fruit extract was found more effective against *Pseudomonas aeruginosa* than Streptomycin at 100% concentration displaying the percentage inhibition value of 115.57±3.51. The leaf extract was found to be least effective among all three extracts exhibiting antibacterial property showing maximum activity against *S. aureus* (99.06±3.01%). The tuber extract was found to be more effective than streptomycin against *S. aureus* and *P. aeruginosa* at 100% concentration (111.28±2.87% of standard inhibition) and above 75%, (116.97±2.65% inhibition at 100 % concentration) respectively. The results obtained are compiled in the Table 1 and Figure 1. A. shows the comparative % bacterial growth inhibition activity of acetone and methanol extracts.

Table 1: Antibacterial activity of different acetone extracts

Part	Conc. (%)	Percent Growth Inhibition by acetone extracts (%)				
		L. monocytogenes	*S. aureus*	*P. aerugenosa*	*S. typhi*	*S. dysenteriae*
Fruit	25%	38.55±0.57	69.31±1.44	61.67±1.00	34.85±1.74	64.45±7.00
	50%	41.36±1.18	75.98±1.63	74.43±2.66	38.30±1.26	65.55±8.00
	75%	46.59±2.00	82.64±1.96	81.53±3.22	44.44±2.50	73.3±6.45
	100%	51.82±2.60	91.30±1.97	108.49±3.00	48.65±1.90	85.55±4.75
Leaf	25%	34.12±1.43	56.63±1.97	-	-	43.87±1.80
	50%	36.95±1.82	63.31±1.96	59.54±2.65	-	50.55±1.98
	75%	39.75±2.05	69.97±1.88	65.20±1.53	-	62.75±4.73
	100%	43.37±3.00	99.06±3.01	74.45±4.59	39.08±1.88	70.53±1.63
Tuber	25%	36.94±3.23	62.63±1.07	77.26±1.16	37.92±3.02	64.43±6.54
	50%	41.35±2.69	75.94±1.88	82.96±3.00	41.37±2.87	65.538.33
	75%	42.97±3.78	91.98±1.63	104.21±3.00	44.06±3.18	73.32±6.44
	100%	53.01±2.48	111.28±2.87	116.97±2.65	51.33±1.36	85.55±4.73

The values represent the % of Streptomycin activity that was exhibited by tested extract
Each data value represents the mean±S.E, n= 6

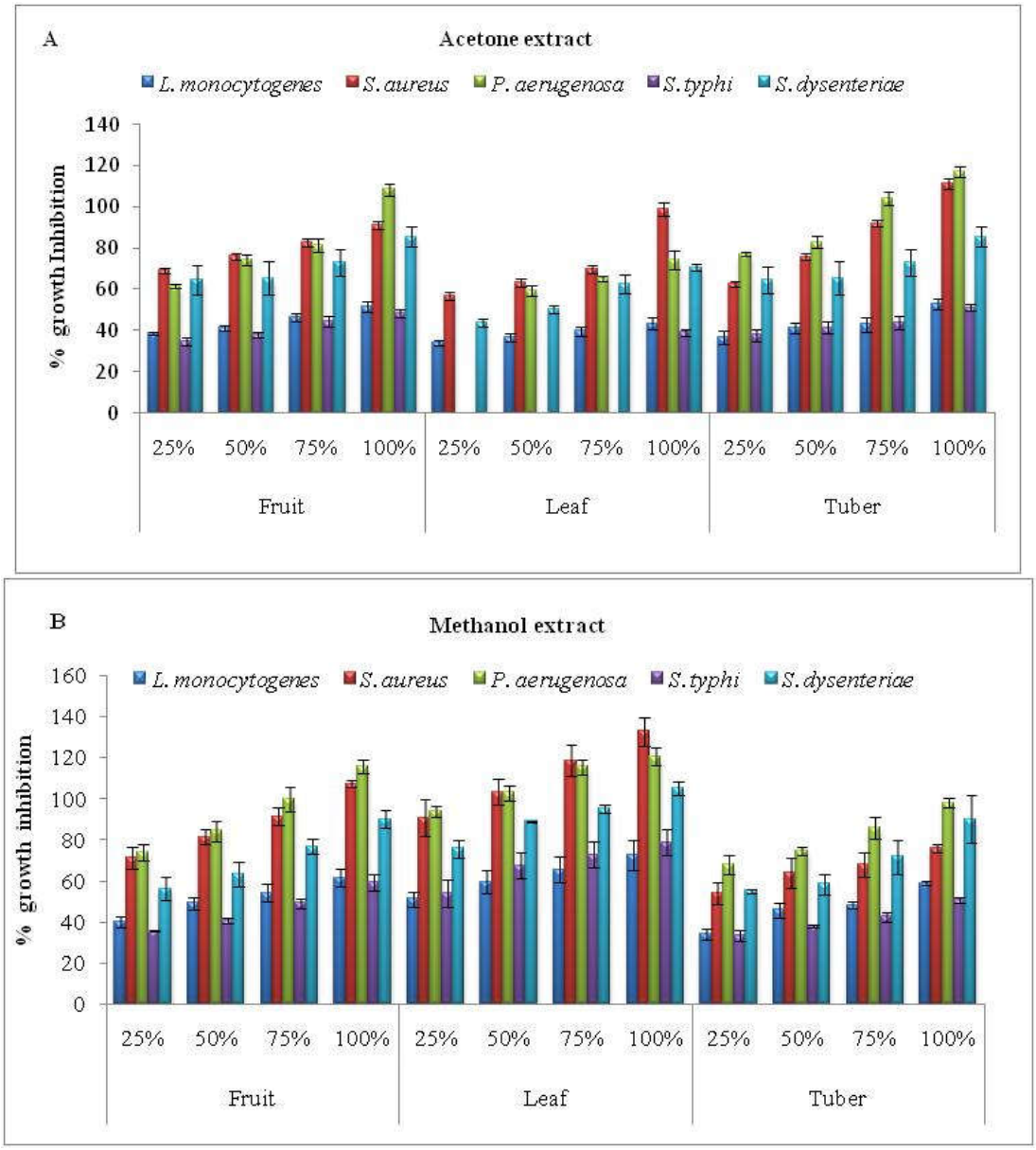


Figure 1: Graphs displaying the percentage of Streptomycin activity that was exhibited by the teste extracts for their antibacterial potential against tested Gram-positive and Gram-negative bacteria (mean ±S.E., n=6). (A.) Acetone extracts. (B.) methanol extract.

B. Methanol extracts

Fruit extract was found to be more effective than standard against *S. aureus* and *P. aerugenosa* at 100% concentration exhibiting 107.30±1.44% and 115.57±3.51% value respectively. Leaf extract exhibited best antibacterial potential against all the tested bacteria. Leaf extract was more effective against *S. aureus, P. aeruginosa* and *S. dysenteriae* than streptomycin above 50% (132.61±6.68% and 120.53±4.72% at 50mg/ml concentration respectively) for first two and at 100% concentration (104.98±3.14%) for the last. Tuber extract has displayed moderate activity. The results obtained are compiled in the Table 2. and Figure 1.B. shows the comparative activity of acetone and methanol extracts.

Table 2: Antibacterial activity of different methanol extracts

Part	Conc. (%)	Percent growth inhibition by methanol extracts (%)				
		L. monocytogenes	*S. aureus*	*P. aerugenosa*	*S. typhi*	*S. dysenteriae*
Fruit	25%	40.15±2.56	71.30±5.20	73.73±4.1	35.62±0.53	56.1±5.69
	50%	48.99±2.92	81.30±3.57	84.36±5.05	40.60±1.36	63.35±6.12
	75%	54.20±4.29	91.30±4.46	99.94±6.01	49.03±2.18	76.67±3.60
	100%	61.46±4.50	107.30±1.44	115.57±3.51	59.37±3.86	89.98±4.15
Leaf	25%	51.133±3.57	90.64±8.95	93.51±2.62	54.01±6.65	75.53±4.46
	50%	59.39±5.72	103.28±6.27	102.81±3.76	67.42±6.47	88.77±0.50
	75%	65.46±6.26	118.64±7.91	115.42±4.52	72.79±6.51	94.98±2.07
	100%	72.30±7.43	132.61±6.68	120.53±4.72	78.529±6.28	104.98±3.14
Tuber	25%	33.73±2.67	53.99±5.22	68.03±4.72	33.31±2.69	54.95±1.15
	50%	45.77±3.76	63.95±7.38	74.40±1.98	37.90±0.79	58.3±4.95
	75%	48.19±1.77	67.97±5.81	85.77±5.61	42.52±2.34	71.65±8.30
	100%	58.64±0.97	75.94±2.15	97.83±2.29	50.55±1.20	89.95±11.65

The values represent the % of Streptomycin activity that was exhibited by tested extract
Each data value represents the mean ±S.E., n=6.

DPPH Radical Scavenging Activity Assay

The antioxidant activity of acetone, distilled water and methanol extracts was investigated using DPPH free radical scavenging assay. Ascorbic acid which was taken as standard has shown 88.89±1.23% DPPH scavenging at the concentration of 100 µg/mL and IC_{50} value of 31.33 µg/mL. Obtained IC_{50} values are tabulated in the Table 3 and Table 4. The plotted graphs are shown in the Figure 2 and 3.

Table 3: DPPH scavenging activity of ascorbate

Conc. of L-Ascorbic acid (µg/mL)	Scavenging activity (%)	IC_{50} value (µg/mL)
20	44.75±1.10	31.33
40	53.09±1.45	
60	66.31±0.90	
80	78.80±1.37	
100	88.89±1.23	

Table 4: DPPH scavenging activity and IC_{50} values of the tested plant extracts

Part	Con. (µg/mL)	Acetone extract		Distilled water extract		Methanol extract	
		Scavenging activity (%)	IC_{50} value (µg/mL)	Scavenging activity (%)	IC_{50} value (µg/mL)	Scavenging activity (%)	IC_{50} value (µg/mL)
Fruit	20	14.74±2.57	60.98	6.47±1.63	60.34	16.59±3.05	65.96
	40	38.88±2.35		25.24±2.44		36.28±4.32	
	60	52.53±3.81		34.08±1.27		47.12±4.10	
	80	64.00±1.99		37.67±1.05		58.92±1.98	
	100	76.22±1.47		40.92±0.88		76.22±1.47	

Leaf	20	17.62±3.07	86.92	6.47±1.63	111.94	23.95±1.33	47.89
	40	37.4±3.39		25.24±2.45		44.58±2.75	
	60	40.44±3.16		34.08±1.27		66.50±3.63	
	80	46.07±3.18		37.67±1.05		78.72±3.50	
	100	53.85±2.33		40.92±0.88		81.30±1.98	
Tuber	20	18.55±1.54	77.132	-	-	13.60±1.63	60.14
	40	34.05±3.63		-		37.96±4.20	
	60	47.30±5.40		-		49.26±1.20	
	80	51.58±3.92		-		70.60±3.63	
	100	63.78±2.11		-		81.06±1.80	

Each data value represents the mean ±S.E, n=6.

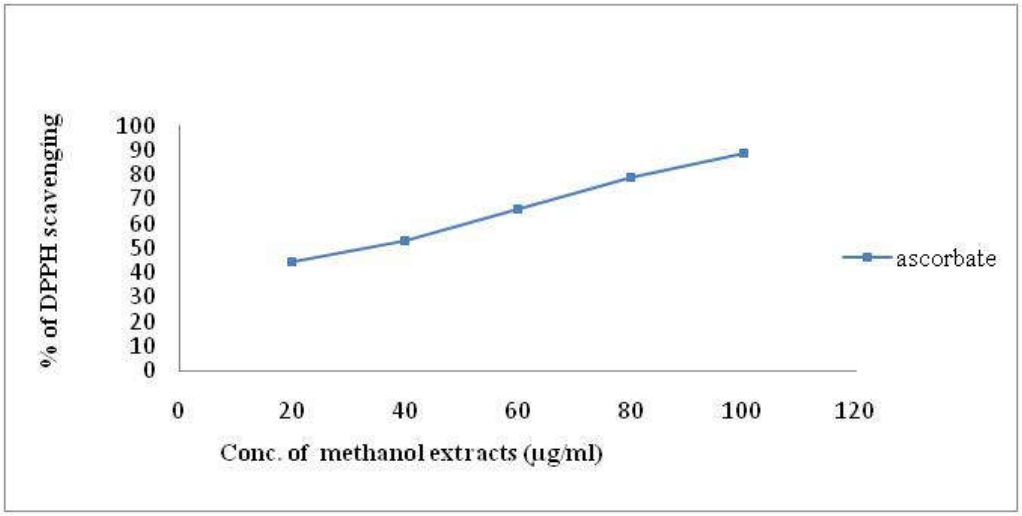
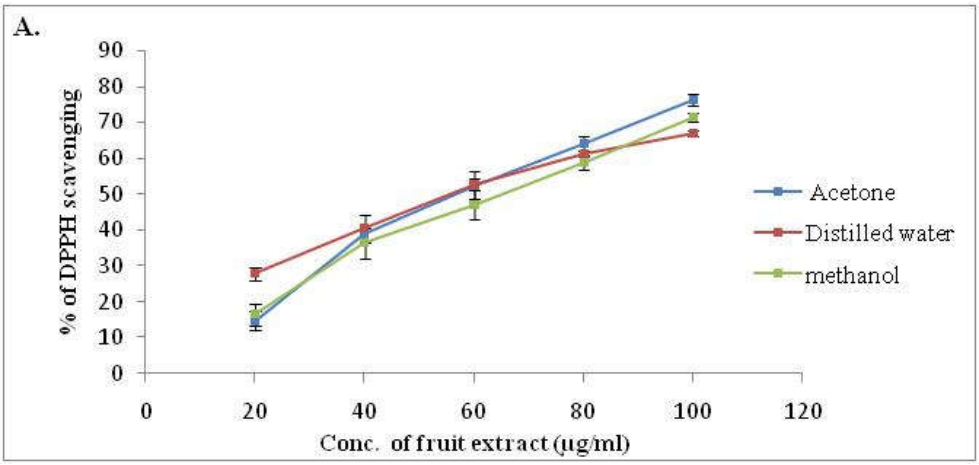


Figure 2: Graph showing DPPH free radical scavenging activity of ascorbate at different concentrations (mean ±SE, n= 6).



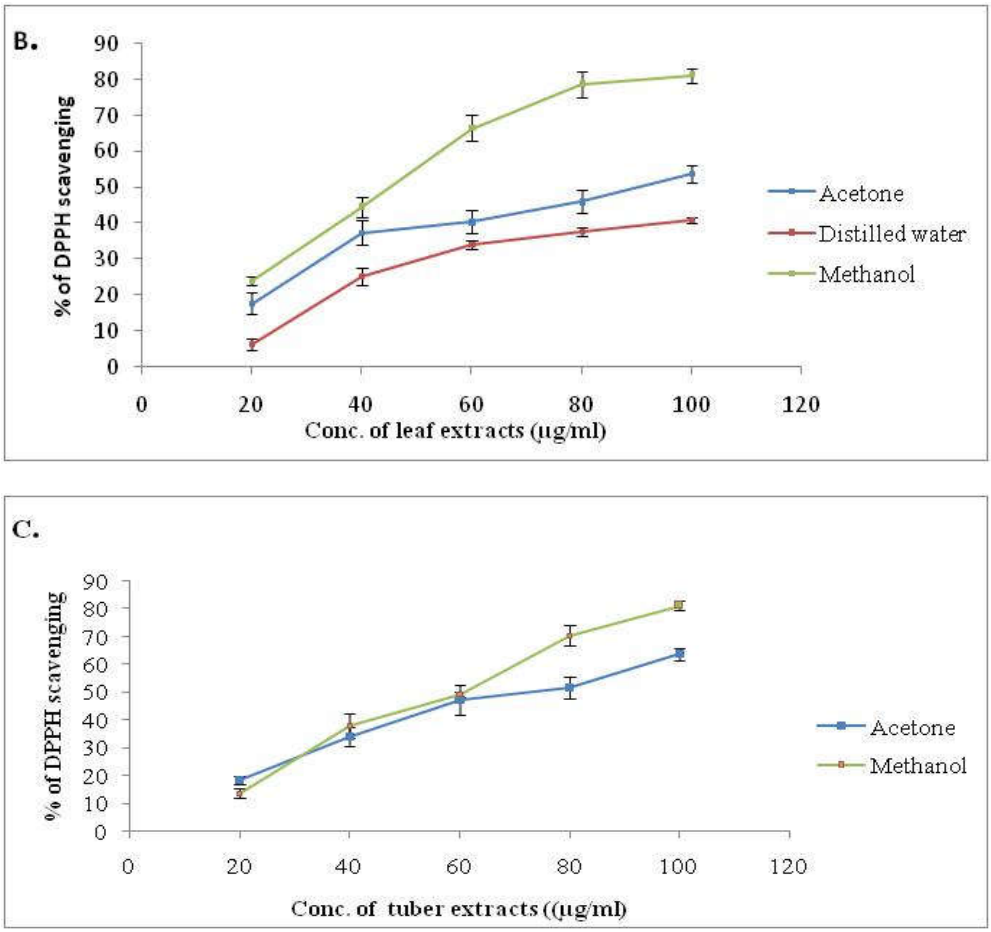


Figure 3: Graph showing comparative antioxidant potential at different concentration. (A.) Fruit extracts, (B.) Leaf extracts and (C.) Tuber extracts. (mean ± SE , n= 6)

A. Fruit extract

The acetone, Distilled water and methanol extracts of fruit have shown good antioxidant property displaying the Percent DPPH scavenging activity of 76.22± 1.47, 40.92±0.88 and 76.22±1.47 % at 100 µg/mL concentration respectively.

B. Leaf extract

Leaf has exhibited maximum DPPH scavenging activity in methanol extract (81.30±1.98 %) followed by acetone (53.85±2.33%) and distilled water extract (40.92±0.88%) at 100 µg/mL concentration.

C. Tuber extract

The acetone and methanol extract of tuber have shown good antioxidant potential with 77.13 and 60.14 µg/mL IC_{50} value, respectively**.** No significant results were exhibited by distilled water extract.

DISCUSSION

Screening of prepared extracts for antibacterial activity

The acetone and methanol extracts of *Arisaema jacquemontii* have shown positive antibacterial results while no result are found in chloroform and distilled water extract. The tuber acetone, fruit methanol and leaf methanol extract were found to be more effective against some the tested bacteria than the standard antibiotic used. Methanol extract of roots was evaluated by Baba and Malik[29] against human pathogenic bacteria. The respective MIC values were calculated and were found to be 0.37 mg/mL for *Staphylococcus aureus* and 0.24 mg/mL for *Salmonella enteritidis.* All other tested bacteria

were also found to have significant sensitivity for methanol root extract. The present investigation matches these results. The tubers of the plants were tested for antibacterial potential by Iqbal *et al.,*[30] using disc diffusion susceptibility assay against six bacterial strains. Moderate activities were recorded for methanol extract at different concentrations against all the tested bacteria. *P. aerugenosa* and *S. aureus* were found to be resistant against the aqueous extract which coincides with the results of present investigation. Secondary metabolites play essential role in free radical scavenging and are also cause of bactericidal assets. Essential oils are among them[31]. The phenolic compounds are partially hydrophobic in nature and are toxic to microbes. The fruits, leaves and roots contain terpenes, saponions and glycosides[32]; roots possess phenols, flavonoids[29] and leaves are reported to contain coumarin, quinins, saponins, tannins, alkaloids, anthraquinon[33] all contributing the antibacterial property of the plant. Cushnie and Lamb[34] (2005) reported antibacterial activity of flavonoids which are involved in the inhibition of nucleic acid synthesis and altering the metabolism.

DPPH Radical Scavenging Activity Assay

In present exploration, among fruit, leaf and tuber, fruit has shown best antioxidant potential. Among these extracts, methanol extracts have shown best results. The lowest IC_{50} value was displayed by methanol leaf extract (47.89 µg/mL) followed by methanol fruit extract (60.14 µg/mL), distilled water fruit extract (60.34 µg/mL), acetone fruit extract (60.98 µg/mL), methanol fruit extract (65.96 µg/mL), acetone tuber extract (77.13 µg/mL), acetone leaf extract (86.92 µg/mL) and distilled water leaf extract (111.94 µg/mL). The antioxidant potential of this plant may be due to high content of phenols, flavonoids, carotenoids terpenes and alkaloids present in it. Most of the oxidising molecules responsible for several diseases are effectively withdrawn by flavonoids[35] by restraining the ROS formation, chelating the trace elements involved in free radical production and regulating the antioxidant defence systems [36]. In the same way, phenols also intensify the oxidative stress tolerance in plants. Sudan *et al.*[33] in 2014 has explored the antioxidant potential of tuber, leaf and fruit of *Arisaema jacquemontii* Blume of north west Himalayan region by using methanol, chloroform and water for FRAP assay. The methanol leaf extract was found to exhibit better chelating capacity and reducing power than fruit and tuber. The results found remarkable chelation power of leaves at 100 µg/mL (58%) as compared to tubers (12%) and fruit extract (34%) In their study, water extract exhibited negligible activity. Roots of *A. jacquemontii* from Uri, Jammu and Kashmir were extracted in methanol to evaluate the antioxidant potential by Baba and Malik[29]. Three assays used in this investigation were DPPH free radical scavenging assay, NBT (Nitroblue- tetrazolium) superoxide anion scavenging assay and FRAP (Ferric Reducing/Antioxidant Power) assay. In all assays, roots have shown significant antioxidant activity.

CONCLUSION

Arisaema jacquemontii is a plant of tremendous possibilities in the field of health and supplements. Great antibacterial activity against *S. aureus, P. aerugenosa* and *S. dysenteriae* in our investigation proves its ethno medical uses for dermatological disorders and microbial infections. Plant has also revealed its antioxidant potential as a good free radical scavenger. The present investigation will form the basis for the further analysis and isolation of its active compounds required prior to usage in medical and food industry.

ACKNOWLEDGEMENT

Authors acknowledge the facilities provided by the Chairman, Department of Biosciences, Himachal Pradesh University, Shimla. First author is also grateful to HPU for baking her financially with HPU-JRF (No. HPU/Biosci/2018/1023).

CONFLICT OF INTEREST

The author declares that there is no conflict of interests regarding the publication of this paper.

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