Antibacterial Activity with Bacterial Growth Kinetics and GC-MS Studies on Leaf and Tuber Extracts of *Arisaema tortuosum* (Wall.) Schott

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ABSTRACT

Background: Pathogenic bacteria (Gram positive/Gram negative) are serving as a vital precursor for the development of infectious diseases in humans. Arisaema tortuosum (Wall.) Schott (ATWS), a famous folklore medicine of Asian region has documented for great medicinal values. Objective: To evaluate the ATWS extracts and their fractions (leaf and tuber part) for antibacterial potential against human pathogens and to probably examine the phytochemicals existing in promising extracts via gas chromatographymass spectroscopy (GC-MS). Methods: The dried young leaves and tubers of ATWS was extracted using $C_{2}H_{2}OH:H_{2}O$ (95:5, v/v) and further fractionated with n-hexane, chloroform, ethyl acetate and butanol, respectively. The antibacterial activity was carried out using agar well diffusion method. Furthermore, the antibacterial kinetic curve of capable extracts/fractions was too studied using time killing assay. Results: Overall, leaves extracts exhibited greater and varying level of Minimum Inhibitory Concentration (MIC)/ Zone of Inhibition (ZOI) against tested bacterial strains: Staphylococcus aureus (Sa), Bacillus subtilis (Bs), Escherichia coli (Ec) and Salmonella typhimurium (St). Additionally, extracts (Ethanolic for St and Bs, n-hexane for Sa and chloroform for Ec) have mostly inhibited the growth of test organisms for approximate 24 hr. The common tentative compounds like phytol, neophytadiene, octadecane, hexahydrofarnesyl acetone etc. were confirmed qualitatively by GC-MS. Conclusion: The antibacterial finding reveals that the leaves of ATWS have considerable antibacterial activity might be due to the presence of various chemical constituents. The tentative compounds confirmed by GC-MS from different extracts may further act as a valuable tool for future researchers against various bacterial illnesses.

Key words: Arisaema tortuosum, Leaf, Tuber, Extracts, Antibacterial activity, GC-MS analysis.

INTRODUCTION

As per WHO guideline, bacteria related infections are serving as vital parameters for the development of large mortality rate in under developing countries. Bacterial contaminations are a noteworthy reason for disorder and passing around the world. The purpose behind it is that a large portion of the pathogens causing enteric infections have created protection from generally utilized medications. Protection of microscopic organisms to anti-microbial expands mortality and extends the stay of patients in doctor's facilities.¹ Addressing the given issue, antimicrobials are especially a concern with negative consequences that incorporate unfavourably hypersensitive responses, repression of the immune system and making the host extremely allergic. Along these lines, it is expected to grow new, more secure, best therapeutic and less expensive anti-infection agents for the treatment of irresistible ailments through herbal remedies. The accomplishment of current medications lies in proceeds with a hunt of new Submission Date: 27-09-2018; Revision Date: 05-01-2019; Accepted Date: 22-04-2019

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medications.² Nature has been as a wellspring of therapeutic agents since ancient times and a great number of present-day medications have been identified from an herbal origin. Globally, numerous herbs have been operated as a promising medicine in day to day life to treat illnesses.³ Arisaema tortuosum (Wall.) Schott (ATWS) commonly known as whipcord cobra lily belonging to plant family Araceae, that has a particular purple or green whip-like spadix, which emerges from the orifice of flower (Jack-in-the-platform) and approximate 30 cm long.⁴ The plant occurrence is ranging from Himalayan forest, China, Myanmar and Southern Indian parts.⁵⁻⁶ Traditionally, a recent report has highlighted that ATWS tuber part claimed for antirheumatic, antiasthmatic, antimicrobial and contraceptive actions. Leaves answered to be reasonably noxious and can be valuable against rheumatism and stomach issue.7 Chemical investigation showed that tuber (Corm) has shown distinctive chemical entities like lectin, n-alkanols, stigmasterol, sitosterol, campesterol, cholesterol, choline chloride and stachhydrine hydrochloride, correspondingly.8 A study in tuber part of ATWS affirmed the presence of flavonoids and prominent role in antioxidant, anti-inflammatory and anticancer applications.9 Out of the constituents, a lectin resulted as principle pro-inflammatory entity and hopeful inhibitor for the human uncontrolled division of cells.¹⁰ Previously, lectins have presented the role in host-pathogen interaction and also the cytotoxic effects on micro-organisms and parasites.¹¹ To the best of our knowledge, mostly tuber part of ATWS was explored for the therapeutic purpose and even leaf part studies were too quite limited.12 Therefore, an attempt was made to screen the promising ATWS antibacterial leaf and tuber extracts and further analyze these extracts for the tentative confirmation of chemical constituents using GC-MS.

MATERIALS AND METHODS

Materials

The whole plant was gathered near to Mandi district, Himachal Pradesh, India in August-September 2013 and authenticated in-house by a CSIR-NISCAIR, New Delhi taxonomist (Reference Number: NISCAIR/RHMD/ Consult/2013/2249/30). Mueller-Hinton (MH) and Mueller-Hinton Agar (MHA) medium were purchased from HiMedia Laboratories. The bacterial strains (*Sa* 11949, *Bs* 441, *Ec* 1687 and *St* 8767), used for culturing were acquired from IMTECH, CSIR laboratory Chandigarh, India and preserved in our aseptic microbiology lab. Resazurin dye was acquired from Sigma-Aldrich Chemie. Supplementary solvents and chemicals were of analytical mark procured from SDFCL, Mumbai, India.

Methods

The leaf and tuber parts of ATWS was cleaned, shade dried, grounded to powder (Coarse) properly. A 250 g of each powdered materials were extracted with ethanol (95:5, v/v) and further fractionated with n-hexane, chloroform, ethyl acetate, butanol and other remained as an aqueous extract. These concentrates were lyophilized and reserved in the dark ($+3^{\circ}$ C to $+4^{\circ}$ C) until further use.

Evaluation of Antibacterial Studies

The culture turbidity of bacteria used for studies was in tune to 0.5 McFarland equivalence prior to use.¹³ Stock solutions of standard ciprofloxacin (0.1 mg/mL) and extracts (20 mg/mL) used for the study were dissolved in 5% DMSO solutions.

Agar well diffusion assay

All bacterial strains were developed on Müeller Hinton Agar (MHA) (HiMedia Laboratories Pvt. Ltd., Mumbai, India) for 24 hr prior to the experiment. The test cultures computing 50 µl were scrubbed/Petri dish of molten Müeller Hinton Agar (MHA) by using an aseptic cotton swab. Wells were prepared using a cork borer (10 mm diameter), filled with the extracts/ciprofloxacin solution (20 mg/mL/1mg/mL) followed by incubation at 37°C for 24 hr. In addition, the blank (5% DMSO) was too examined against the tested bacterial strains.¹⁴

Minimum Inhibitory Concentration (MIC) determination

A minor revision to broth micro dilution bioassay was done for calculating MIC value.¹⁵ In brief, resazurin tablet (270 mg) was suspended in sterile distilled water (40 mL), followed by swirling in order to guarantee a welldissolved and homogenous solution. Additionally, 50 μ L of extracts and standard (20 mg/mL/1 mg/mL) were added into multi-well microplate reader holding 50 μ L of Muller-Hinton broth, 5 μ L bacterial cultures and 5 μ L resazurin, respectively. Resazurin dye pink color positively correlates with bacterial growth. The microplate was clogged with a lid and incubated for 24 hr at 37°C.

Bacterial growth kinetics

Bacterial growth period with test extracts was determined by measuring the OD_{625} in ELISA microplate reader (BiotekEL x 800) and further bacterial inhibition rate was analyzed up to 48 hr.¹⁶

GC-MS analysis

The separating course of action was carried out using GC-MS (QP2010S; Shimadzu, Kyoto, Japan). Around 0.1000% ppm solution of promising antibacterial extracts was prepared and 0.001 mL of individual

extract was inserted using Rxi-5Sil MS column. Helium gas was utilized as a carrier gas with a flow rate of 1 mL/min. The ion source of capacity 250°C with ionization energy 70 eV was used. Confirmation was done by comparing mass spectra of analyzed components with reference mass spectra of NIST 11 and Willey 8 library.^{17,18}

Statistical Analysis

The results were manifested as the triplicate measurements of Mean±Standard Deviation (SD) against tested bacterial strains.

RESULTS

The *in-vitro* antibacterial potential of ATWS extracts was tested by ZOI and MIC studies. The MIC (Leaves and tubers extracts) ranged from 78.13×10^{-3} to 2500×10^{-3} mg/mL while the ZOI ranged between 7.00 to 11.00 mm and the results are summarized in Table 1. The promising leaf antibacterial extract/fractions of ATWS (Ethanol, n-hexane and chloroform) were studied for bacterial kinetic growth patterns for a period up to 48 hr. Figure 1 depicts the time-dependent inhibitory effect of capable ATWS extracts against four foodborne microorganisms (Bs, Ec, Sa and St). Overall, ethanol leaf extract inhibited the maximum growth of Bs and St for approximate 24 hr compared with other extracts. Additionally, n-hexane leaf fraction showed inhibition in growth for Sa while chloroform (Leaf fraction) confirmed slow down the growth against Ecover 24 hr (Figure 1). Furthermore, the noteworthy plant extracts (Ethanolic, n-hexane and chloroform)



Figure 1: The Time-Dependent Inhibitory Effect of ATWS Extracts/Fractions (Ethanolic, n-hexane and Chloroform) against *B. subtilis, E. coli, S. aureus* and *S. typhimurium.*

were analyzed by GC-MS. The presence of chemical entities was affirmed by noting the similarity of tested components with standard mass spectra of NIST and Willey library. In the GC-MS investigation of ATWS, sixteen compounds were confirmed in ethanolic leaf extract, twenty-one compounds in n-hexane fraction while twenty seven in chloroform fraction. The active chemical entities with their R. Time (RT), Area, Area%, Height, Height%, name and Base ion m/z in ethanolic, n-hexane and chloroform extracts of ATWS are summarized in Table 2, Table 3 and Table 4, respectively. Chromatogram of ethanolic, n-hexane and chloroform extracts are presented in Figure 2 (a, b, c). The chief chemical entities which indicated in ethanolic extract are phenol, 2,4-bis(1,1-dimethylethyl)- (6.93%), 3-heptadecanol (0.56 %), pentadecanoic acid, methyl ester (0.58%), neophytadiene (0.94), hexahydrofarnesyl acetone (1.11%), methyl hexadec-9-enoate (1.69%), hexadecanoic acid, methyl ester (19.48%), 9,12-octadecadienoic acid, methyl ester (20.70%), 9,12,15-octadecatrienoic acid, methyl ester, (z,z,z)- (37.96%), cis-13-octadecenoic acid, methyl ester (1.10%), phytol (3.27%), hexadecane, 2,6,10,14-tetramethyl-(0.60%), methyl stearate (2.95%), octadecane, 1-chloro- (0.56%), docosanoic acid, methyl ester (1.00%) and tetracosanoic acid, methyl ester (0.56%). In n-hexane fraction, twenty one compounds are 2,4-ditert-butylphenol (5.69%), hexadecan (1.76%), octadecane (5.25%), hexahydrofarnesyl acetone (7.80%), cyclopropanenonanoic acid, 2-[(2-butylcyclopropyl) methyl]-, methyl ester (1.05%), hexadecanoic acid, ethyl ester (13.71%), 9,12-octadecadienoic acid, methyl ester (1.60%), 9,12,15-octadecatrienoic acid, methyl ester,





Table 1: Minimum inhibitory concentration and zone of inhibition of ATWS extracts for tested bacterial strains.								
Plant Materials	MIC (mg/ml)/Zone of Inhibition (diameter in mm)							
	Sa	Bs	Ec	St				
А	312.5×10 ⁻³ /7±0.3	1250×10-3/11±0.7	2500×10-3/7±0.3	1250×10-3/11±0.4				
В	2500×10-3/6±0.6	2500×10-3/6±0.8	2500×10-3/6±0.8	1250×10-3/5±0.2				
A1	78.13×10-3/9±0.4	2500×10-3/10±0.9	2500×10-3/10±0.3	2500×10-3/10±0.7				
B1	156.25×10-3/8±0.5	2500×10-3/9±0.3	1250×10-3/5±0.3	1250×10-3/6±0.3				
A2	156.25×10-3/7±0.6	2500×10-3/10±0.4	2500×10-3/11±0.8	1250×10-3/9±0.4				
B2	312.5×10 ⁻³ /5±0.3	2500×10-3/8±0.4	1250×10-3/8±0.4	1250×10-3/6±0.3				
A3	78.13×10 ⁻³ /5±0.3	1250×10-3/7±0.8	1250×10-3/6±0.2	1250×10-3/9±0.5				
B3	78.13×10 ⁻³ /5±0.5	1250×10-3/6±0.4	1250×10-3/5±0.1	1250×10-3/7±0.3				
A4	1250×10-3/5±0.3	2500×10-3/6±0.8	1250×10-3/5±0.3	2500×10-3/-				
B4	2500×10-3/5±0.4	2500×10-3/5±0.6	1250×10-3/5±0.7	2500×10-3/-				
A5	2500×10-3/5±0.1	2500×10-3/5±0.2	2500×10-3/5±0.3	5000×10-3/-				
B5	2500×10-3/5±0.4	2500×10-3/5±0.3	2500×10-3/5±0.7	2500×10-3/-				
S	0.78×10 ⁻³ /27±0.3	1.56×10 ⁻³ /23±0.2	6.25×10 ⁻³ /29±0.4	0.09×10 ⁻³ /25±0.3				

A/B-ethanolic extract (leaves/tubers), A1/B1-hexane extract (leaves/tubers), A2/B2-chloroform extract (leaves/tubers), A3/B3-ethyl acetate extract (leaves/tubers), A4/B4-butanolic extract (leaves/tubers), A5/B5-aqueous extract (leaves/tubers), S-standard (ciprofloxacin), Sa-Staphylococcus aureus, Bs-Bacillus subtilis, Ec-Escherichia coli, St-Salmonella typhimurium; Zone of inhibition: used concentration of extracts/standard-20/0.1 mg/ml. Each value is expressed as mean ± SD

Table 2: Chemical Entities Detected in ATWS Ethanolic Extract.								
R. Time	Area	Area%	Height	Height%	Name	Base m/z		
17.334	6716331	6.93	1670893	5.71	Phenol, 2,4-Bis(1,1-dimethylethyl)-	191.20		
24.026	547481	0.56	193571	0.66	3-Heptadecanol	59.10		
24.406	564046	0.58	182908	0.62	Pentadecanoic acid, Methyl ester	74.10		
24.682	912938	0.94	310697	1.06	Neophytadiene	68.10		
24.787	1074728	1.11	355874	1.22	Hexahydrofarnesyl acetone	58.05		
26.364	1641133	1.69	523825	1.79	Methyl hexadec-9-enoate	55.05		
26.492	18894249	19.48	6021238	20.57	Hexadecanoic acid, Methyl ester	74.10		
29.741	20072235	20.70	5894362	20.14	9,12-Octadecadienoic acid, Methyl ester	67.10		
29.871	36805957	37.96	10986375	37.53	9,12,15-Octadecatrienoic acid, Methyl ester, (z,z,z)-	79.10		
29.954	1070929	1.10	334764	1.14	Cis-13-octadecenoic acid, Methyl ester	55.05		
30.051	3167313	3.27	970896	3.32	Phytol	71.10		
30.242	579115	0.60	144922	0.50	Hexadecane, 2,6,10,14-tetramethyl-	57.10		
30.332	2859063	2.95	1007501	3.44	Methyl stearate	74.05		
33.967	544722	0.56	118980	0.41	Octadecane, 1-chloro-	57.10		
37.160	973982	1.00	363648	1.24	Docosanoic acid, Methyl ester	74.05		
40.238	547020	0.56	192723	0.66	Tetracosanoic acid, Methyl ester	74.10		

(z,z,z)- (2.54%), phytol (16.15%), ethyl 9,12-hexadecadienoate (8.35%), ethyl (9z,12z)-9,12-octadecadienoate (14.50%), 9-octadecenoic acid (z)-, ethyl ester (0.70%), octadecanoic acid, ethyl ester (1.83%), phytol, acetate (0.82%), heptacosane (0.77%), ethyl nonadecanoate (0.74%), 1-octadecanol (1.82%), eicosane (3.24%), tetracosylheptafluorobutyrate (5.07%) and 1-heptacosanol (5.46%). Twenty seven compounds (chloroform) which are indicated by GC-MS are phenol, 2,4-bis(1,1dimethylethyl)- (8.68%), hexadecane (1.86%), (-)-loliolide (1.49%), e-15-heptadecenal (1.15%), nonadecane (2.98%), 3-heptadecanol (0.94%), isopropyl myristate (0.37%), neophytadiene (2.86%), hexahydrofarnesyl acetone (2.74%), phytol, acetate (1.17%), 1,2-benzenedicarboxylic acid, bis (2-methylpropyl) ester (13.11%), tetrapentacontane, 1,54-dibromo- (0.35%), phthalic

Table 3: Chemical Entities Detected in ATWS n-hexane Fraction.								
R. Time	Area	Area%	Height	Height%	Name	Base m/z		
17.746	4768387	5.69	1115745	4.50	2,4-Ditert-butylphenol	191.15		
19.906	1476398	1.76	472240	1.90	Hexadecan	57.10		
24.346	954965	1.14	315176	1.27	Octadecane	57.05		
25.112	4404683	5.25	1337043	5.39	Neophytadiene	68.10		
25.218	6539733	7.80	2034021	8.20	Hexahydrofarnesyl acetone	58.05		
25.613	878438	1.05	262524	1.06	Cyclopropanenonanoic acid, 2-[(2-Butylcyclopropyl)methyl]-, Methyl ester	149.10		
28.253	11491970	13.71	3622566	14.60	Hexadecanoic acid, Ethyl ester	88.10		
30.164	1343093	1.60	441804	1.78	9,12-Octadecadienoic acid, methyl ester	67.05		
30.278	2130909	2.54	654523	2.64	9,12,15-Octadecatrienoic acid, Methyl ester, (z,z,z)-	79.10		
30.516	13534814	16.15	4194714	16.90	Phytol	71.10		
31.401	7001340	8.35	2138446	8.62	Ethyl 9,12-hexadecadienoate	67.10		
31.519	12154625	14.50	3714895	14.97	Ethyl (9z,12z)-9,12-octadecadienoate	79.10		
31.633	587962	0.70	173840	0.70	9-Octadecenoic acid (z)-, ethyl ester	55.05		
31.990	1535227	1.83	498854	2.01	Octadecanoic acid, Ethyl ester	88.10		
32.349	691102	0.82	206014	0.83	Phytol, Acetate	68.10		
33.872	645185	0.77	216792	0.87	Heptacosane	57.10		
35.431	617910	0.74	200934	0.81	Ethyl nonadecanoate	88.10		
37.081	1528344	1.82	461910	1.86	1-Octadecanol	57.10		
37.178	2716157	3.24	827885	3.34	Eicosane	57.10		
40.247	4253016	5.07	1088943	4.39	Tetracosylheptafluorobutyrate	57.10		
43.952	4575755	5.46	841134	3.39	1-Heptacosanol	57.10		

acid, butyl oct-3-yl ester (4.55%), dibutyl phthalate (20.72%), phthalic acid, 5-methylhex-2-yl butyl ester (9.01%), hexadecanoic acid, ethyl ester (3.67%), eicosane (5.06%), phytol (1.67%), dotriacontane (0.95%), octadecanoic acid, methyl ester (0.34%), 1-heneicosanol (1.26%), heneicosane (3.16%), tetrapentacontane (0.59%), octadecane, 3-ethyl-5-(2-ethylbutyl)- (0.53%), n-tetracosanol-1 (0.93%), celidoniol, deoxy- (2.07%) and 1,2-benzenedicarboxylic acid (7.77%). The maximum number for tentative confirmation of chemical entities was identified in chloroform fraction while minimum probable chemical components were found in ethanolic extract.

DISCUSSION

In the current era, the enthusiasms toward the investigation of the herbal chemical entities and their actions have enlarged. The GC–MS is a powerful skill for qualitative and quantitative examination of bioactive constituents in a plant. Decent variety of plants and herbs holding different phytoconstituents with therapeutic spectrum can be operated as a promising remedial key. Distinctive phytochemicals have been found to have an expansive scope of activities, which may help in insurance for serious infections.¹⁹ The common chemical entities found in all three extracts/fractions (Ethanolic, n-hexane and chloroform) are phenol/phenol analogue (6.93% in ethanolic, 5.69% i.e. 2,4-ditert-butylphenol in n-hexane and 8.68% in chloroform), phytol/phytol analogue (3.27% in ethanolic, 16.15% in n-hexane and 1.67% in chloroform), neophytadiene (0.94% in ethanolic, 5.25% in n-hexane and 2.86% in chloroform), octadecane (0.56% in ethanolic, 1.14% in n-hexane and 0.53% in chloroform), hexahydrofarenesyl acetone (1.11% in ethanolic, 7.80% in n-hexane and 2.74% in chloroform), methyl ester (1.10% in ethanolic, 1.05% in n-hexane and 0.34% in chloroform) and hexadecanoic acid (19.48% in ethanolic, 13.71% in n-hexane and 3.67% in chloroform). The GC-MS studies of promising ATWS extracts/fractions (Ethanolic, n-hexane and chloroform) revealed numerous chemical constituents which have many assets. The compound phytol which is indicated in GC-MS studies has known for its antimicrobial, anticancer, anti-inflammatory and cosmetics applications.^{20,21} Moreover, constituent 2,4-ditert-butylphenol has reported on antioxidant and antimicrobial properties while neophytadiene in excellent analgesic, antipyretic, anti-inflammatory, antimicrobial and anti-

Table 4: Chemical Entities Detected in ATWS Chloroform Fraction.								
R. Time	Area	Area%	Height	Height%	Name	Base m/z		
17.034	11597563	8.68	2797907	7.02	Phenol, 2,4-bis(1,1-dimethylethyl)-	191.15		
19.188	2489368	1.86	773362	1.94	Hexadecane	57.10		
23.122	19919001	1.49	461252	1.16	(-)-Loliolide	11.10		
23.459	1541289	1.15	509796	1.28	E-15-Heptadecenal	55.05		
23.610	3979223	2.98	1422204	3.57	Nonadecane	57.05		
23.713	1259473	0.94	396501	0.99	3-Heptadecanol	59.05		
24.080	496366	0.37	177876	0.45	Isopropyl myristate	60.00		
24.365	3824997	2.86	1325483	3.33	Neophytadiene	68.10		
24.471	3665626	2.74	1289674	3.24	Hexahydrofarnesyl acetone	58.05		
25.246	1557489	1.17	475247	1.19	Phytol, Acetate	82.05		
25.823	17511494	13.11	4827645	12.11	1,2-Benzenedicarboxylic acid, Bis(2- methylpropyl) ester	149.05		
26.058	468030	0.35	125843	0.32	Tetrapentacontane, 1,54-dibromo-	57.10		
26.156	6078595	4.55	2030212	5.09	Phthalic acid, Butyl oct-3-yl ester	149.05		
26.798	27682239	20.72	7705748	19.33	Dibutyl phthalate	149.05		
27.131	12042290	9.01	3654688	9.17	Phthalic acid, 5-methylhex-2-yl butyl ester	149.05		
27.498	4904630	3.67	890482	2.23	Hexadecanoic acid, Ethyl ester	149.05		
27.650	6756050	5.06	1999901	5.02	Eicosane	57.10		
29.713	2227962	1.67	699717	1.76	Phytol	71.10		
29.910	1262927	0.95	457304	1.15	Dotriacontane	57.05		
30.000	453431	0.34	174634	0.44	Octadecanoic acid, Methyl ester	74.05		
31.232	1689121	1.26	605386	1.52	1-Heneicosanol	57.05		
31.340	4224869	3.16	1620539	4.07	Heneicosane	57.10		
33.586	788103	0.59	311416	0.78	Tetrapentacontane	57.10		
33.895	706242	0.53	230962	0.58	Octadecane, 3-Ethyl-5-(2-ethylbutyl)-	57.05		
34.653	1238690	0.93	455164	1.14	N-Tetracosanol-1	57.10		
34.740	2770215	2.07	1040339	2.61	Celidoniol, Deoxy-	57.05		
36.850	10375067	7.77	3399575	8.53	1,2-Benzenedicarboxylic acid	149.05		

oxidant spectrum.²²⁻²³ Previous studies on octadecane derivative showed the moderate inhibitory effect against bacterial strains.²⁴ Another study of oil comprising of hexahydrofarnesyl acetone constituent has reported for its antimicrobial effect.²⁵ The fatty acid (Methyl ester) reported for moderate antimicrobial activities.²⁶ The leaves extracts showed varying marked antibacterial worth which might be owing to the supply of active phytoconstituents in the specific fraction of ATWS. The greater antibacterial activity might exhibit due to the presence of more than one active constituent soluble in a promising fraction.²⁷ This may also act as a powerful tool to further assess their usage in food and non-food system.

CONCLUSION

In the present investigation, leaves part of varying extracts has revealed the significant antibacterial prospective against tested bacterial strains. The estimation of the level of bacterial resistance to antibiotics serves as a vital precursor for management of infectious diseases. Thus, kinetic studies for evaluation of antibiotic susceptibility have presented numerous merits such as predicting concentration accurately, long antibiotic exposure examinations etc.²⁸ In order to further understand the chemical nature guided for these activities, it may act as a better understanding tool to explore further these results.

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CONFLICT OF INTEREST

The authors declare no conflict of interst.

ABBREVIATIONS

ATWS: Arisaema tortuosum (Wall.) Schott; **Sa:** Staphylococcus aureus; **Bs:** Bacillus subtilis; **Ec:** Escherichia coli; **St:** Salmonella typhimurium; **MIC:** Minimum Inhibitory Concentration; **ZOI:** Zone of Inhibition; **GC-MS:** gas chromatography-mass spectrmetry; **MH:** Mueller-Hinton; **MHA:** Mueller-Hinton Agar.

REFERENCES

- Winstanley TG, Limb DI, Eggington R, Hancock F. A 10-year survey of the antimicrobial susceptibility of urinary tract isolates in the UK: The Microbe Base project. J Antimicrob Chemother. 1997;40(4):591-4.
- Agrawal P, Rai V, Singh RB. Randomized placebo-controlled, single blind trial of holy basil leaves in patients with noninsulin-dependent diabetes mellitus. Int J Clin Pharmacol Ther. 1996;34(9):406-9.
- Kiruthika KA, Jaisheeba A, Sornaraj R. Evaluation of antibacterial activity of some selected Angiosperm flower extract. Int J Chemtech Res. 2011;3(4):1945-51.
- Pragada PM, Rao DS, Venkaiah M. Study of some ethnomedicinal plants for treatment of dysentery of North Coastal Andhra Pradesh, India. Int J Biosci. 2012;2(1):18-24.
- Choudhary K, Singh M, Pillai U. Ethnobotanical survey of Rajasthan-An update. Am Eurasian J Bot. 2008;1(2):38-45.
- Sharma PP, Majumdar AM. Traditional knowledge of plants from Toranmal Plateau of Maharashtra. IJTK. 2003;2(3):292-6.
- Verma H, Lal V, Pant K, Soni N. A Ethno medicinal Review on Arisaema tortuosum. Int J Adv Pharm Biol Chem. 2012;1(2):176-9.
- Milgani BD, Cxhawla AS, Singh A, Gaind KN. Chemical investigation of Arisaema tortuosum corm. IJP. 1978;40(1):24-5.
- Nile SH, Park SW. HPTLC analysis, antioxidant, anti-inflammatory and antiproliferative activities of *Arisaema tortuosum* tuber extract. Pharm Biol. 2014;52(2):221-7.

- Dhuna V, Bains JS, Kamboj SS, Singh J, Saxena AK. Purification and characterization of a lectin from *Arisaema tortuosum* Schott having *in-vitro* anticancer activity against human cancer cell lines. J Biochem Mol Biol. 2005;38(5):526-32.
- Iordache F, Ionita M, Mitrea LI, Fafaneata C, Pop A. Antimicrobial and antiparasitic activity of lectins. Curr Pharm Biotechnol. 2015;16(2):152-61.
- Kant K, Lal UR, Ghosh M. In silico prediction and wet lab validation of Arisaema tortuosum (Wall.) schott extracts as antioxidant and anti-breast cancer source: A comparative study. Pharmacogn Mag. 2017;13(52):786-90.
- Rojas JJ, Ochoa VJ, Ocampo SA, Muñoz JF. Screening for antimicrobial activity of ten medicinal plants used in Colombian folkloric medicine: A possible alternative in the treatment of non-nosocomial infections. BMC Complement Altern Med. 2006;6(1):2.
- Wong MH, Lim LF, Ahmad BF, Assim BZ. Antioxidant and antimicrobial properties of *Litseaelliptica* Blume and *Litsearesinosa* Blume (*Lauraceae*). Asian Pac J Trop Biomed. 2014;4(5):386-92.
- Sarker SD, Nahar L, Kumarasamy Y. Microtitre plate-based antibacterial assay incorporating resazurin as an indicator of cell growth and its application in the *in vitro* antibacterial screening of phytochemicals. Methods. 2007;42(4):321-4.
- Kumar VD, Verma PR, Singh SK. Morphological and *in vitro* antibacterial efficacy of quercetin loaded nanoparticles against food-borne microorganisms. LWT-Food Sci Technol. 2016;66:638-50.
- 17. Abirami P, Rajendran A. GC-MS analysis of methanol extracts of *Vernoniacinerea*. Euro J Exp Biol. 2012;2(1):9-12.
- Prajapati R, Roy S, Mishra S, Raza SK, Thakur LK. Formulation development, standardization and antimicrobial activity of *Ageratum conyzoides* extracts and their formulation. Int J Pharm Pharm Sci. 2014;6(2):369-74.
- 19. Liu RH. Health benefits of fruits and vegetables are from additive and synergic combinations of phytochemicals. Am J Clin Nutr. 2003;78(3):517-20.
- Grover N, Patni V. Phytochemical characterization using various solvent extracts and GC-MS analysis of methanolic extract of *Woodfordiafruticosa* kurz. Int J Pharm Pharm Sci. 2013;5(4):291-5.
- Ginty MD, Letizia CS, Api AM. Fragrance material review on phytol. Food Chem Toxicol. 2010;48(3):59-63.
- Varsha KK, Devendra L, Shilpa G, Priya S, Pandey A, Nampoothiri KM. 2, 4-Di-tert-butyl phenol as the antifungal, antioxidant bioactive purified from a newly isolated *Lactococcus* sp. Int J Food Microbiol. 2015;211:44-50.
- Raman BV, La S, Saradhi MP, Rao BN, Khrisna AN, Sudhakar M, et al. Antibacterial, antioxidant activity and GC-MS analysis of *Eupatorium* odoratum. Asian J Pharm Clin Res. 2012;5(2):99-106.
- Nguyen HT, Ho DV, Vo HQ, Le AT, Nguyen HM, Kodama T, et al. Antibacterial activities of chemical constituents from the aerial parts of *Hedyotispilulifera*. Pharm Biol. 2017;55(1):787-91.
- Radulović N, Stojanović G, Palić R. Composition and antimicrobial activity of Equisetum arvense L. essential oil. Phytother Res. 2006;20(1):85-8.
- Chandrasekaran M, Kannathasan K, Venkatesalu V. Antimicrobial activity of fatty acid methyl esters of some members of Chenopodiaceae. Z Naturforsch C. 2008;63(5-6):331-6.
- Bibi Y, Nisa S, Chaudhary FM, Zia M. Antibacterial activity of some selected medicinal plants of Pakistan. BMC Complement Altern Med. 2011;11(1):52.
- Theophel K, Schacht VJ, Schlüter M, Schnell S, Stingu CS, Schaumann R, *et al.* The importance of growth kinetic analysis in determining bacterial susceptibility against antibiotics and silver nanoparticles. Front Microbiol. 2014;5:544.

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PICTORIAL ABSTRACT



ABOUT AUTHORS

SUMMARY

- *Arisaema tortuosum* (Wall.) Schott (ATWS), commonly known as whipcord cobra lily (family: Araceae) has great therapeutic applications.
- Very few studies have been documented on ATWS (tubers) such as antioxidant, anti-inflammatory, antimicrobialand anticancer properties.
- Our objectives were to explore limited studies as evaluation of the ATWS different extracts and their fractions (leaves and tubers) for antibacterial activity and to tentatively analyze the components present in promising extracts using Gas Chromatography-Mass Spectroscopy (GC-MS).
- Overall, leaf extract and its fractions showed remarkable activity and kinetic pattern against *Staphylococcus aureus, Bacillus subtilis, Escherichia coli* and *Salmonella typhimurium*.
- Numerous tentative compounds were qualitativelyindicated in promising antibacterial extracts using GC-MS.



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