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Screening of successive extracts of *Amorphophallus konjac* for antibacterial activity

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Amorphophallus **is an Aroid family member, native to Asia.** ***Amorphophallus konjac*** **K. Koch ex N.E.Br. is also known as snake plant due to snake like outlines on its stem. In Mount Abu the plant is grown in wild and known for its toxic principles. In Traditional Chinese System of Medicine (TCM), it was mentioned that gel prepared from flour has been used in detoxification, tumor suppression, phlegm liquefaction and skin disorders. In the present research work, attempts were made to authenticate and validate the ethno-medicinal potentials of** ***A. konjac*** **antimicrobial activity which could be alternate for current synthetic antimicrobial agents. The plant was selected for screening of antimicrobial efficacy against eight selected bacterial strains viz.** ***Staphylococcus aureus*** **(ATCC- 2921),** ***Klebsiella pneumoniae*** **(ATCC 700603),** ***Enterobacter cloacae*** **(ATCC 13047),** ***Escherichia coli*** **(ATCC 25922),** ***Staphylococcus aureus*** **(ATCC 25923),** ***Proteus mirabilis*** **(ATCC 12453),** ***Enterobacter cloacae*** **(ATCC-13047),** ***Enterococcus faecalis*** **(ATCC 29212) and** ***Streptococcus pneumoniae*** **(ATCC 6305). The results of antimicrobial activity of crude dicholormethane (DCM), ethyl acetate, chloroform and methanol were significant. DCM extract C (10 mg/disc) possess maximum efficacy against** ***S. pneumoniae*** **(IZ = 20 mm; AI = 1.25). The main cause of community acquired pneumonia and septicemia in HIV infected patients is caused by** ***S. pneumoniae*** **microorganism. Further, bioactivity guided fractionation of pure compounds from DCM extract of** ***A. konjac*** **can lead to work as novel antibiotic in future. Therefore, the extract can also be used for isolation of volatiles compounds with potentials so that the extract / active fraction / pure compounds can be used as nasal spray in future therapeutics.**

Key words: *Amorphophallus konjac*, antibacterial activity, antimicrobial agents, ethnomedicinal plant.

INTRODUCTION

Amorphophallus konjac possess vast history of consumption in tropical and subtropical Asia as a potentials source of food and also included in Traditional Chinese Medicine (TCM) (Liu et al., 1998).

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Abbreviations: ATCC, American type culture collection; **TCM,** traditional Chinese system of medicine; **DCM,** dicholormethane; **NAM,** nutrient agar medium; **AI,** activity index.

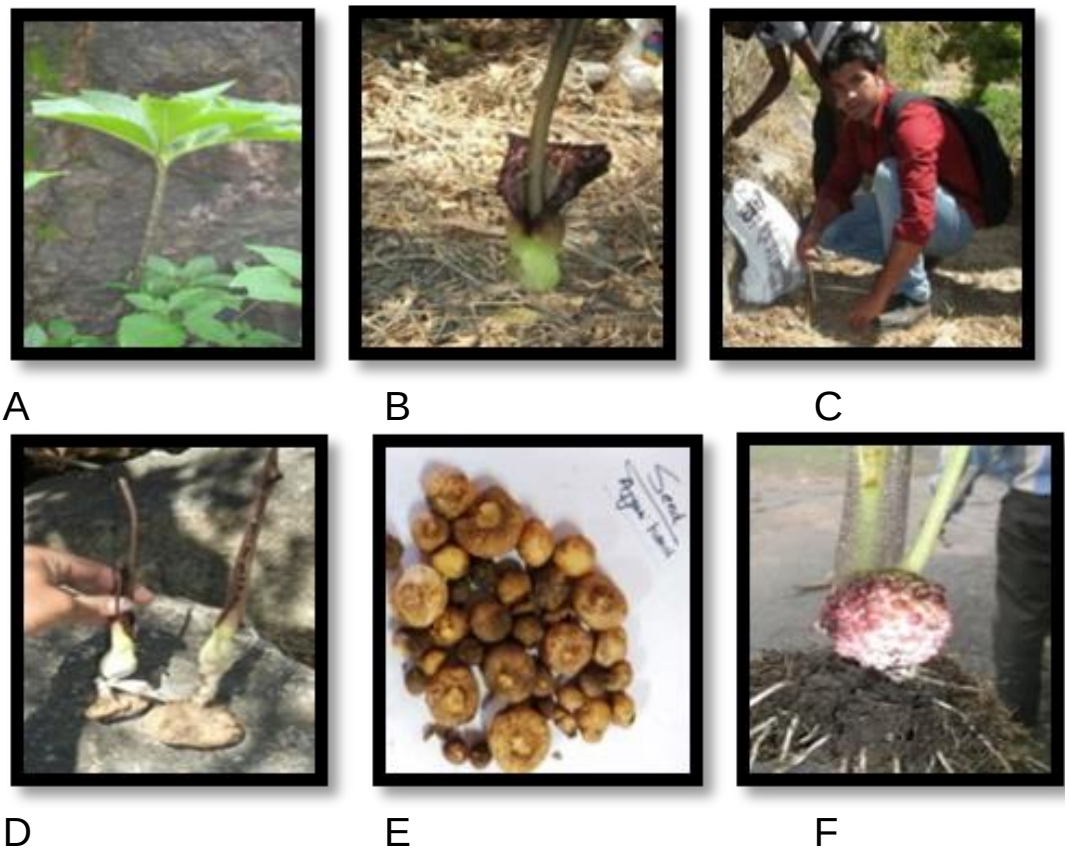

Figure 1. Different plant parts of *Amorphophallus konjac*. **A.** Whole plant; **B and D.** Mature stage. **C.** Research scholar collecting of plant material. **E.** Rhizome of plant. **F.** Root.

Amorphophallus is a genus of Aroid family and native to Asia. *Amorphophallus campanulatus sp*. showed antimicrobial efficacy against *Pseudomonas aeruginosa* and very less efficacy against *K. pneumonia* (Pandey and Gupta, 2013). *A. campanulatus* is used as fibrous diet in Indian food and locally known as *suran*. Ethnomedicinal background of *A. campanulatus* showed that it can cure snake bite which is used by tribal people of Rajasthan (Jain et al., 2005; Kavitha et al., 2011). *A. konjac* is also known as Snake Plant due to its morphology of snake like outlines on stem. It originates in South East Asia. They are recurrent plants with an alternative stem in the shape of a tuber and a highly dissect umbrella-shaped leaf sharp edge (Hetterscheid and Ittenbach, 1996). *Amorphophallus rivieri* is a synonym for *A. konjac* and it is commonly known as *devil's* tongue, snake palm or voodoo lily by local people. It has been cultivated in China for more than 2000 years (Long, 1998). Whole corm extract of this species have been used as a TCM for the treatment of asthma, cough, hernias, breast pain, burns and skin disorders (Niwa et al., 2010). Moreover, the corm tissues are known to be an important foundation of glucomannan, a soluble, non-cellulosic polysaccharide (Takigami, 2000).

Currently, konjac is grown in China, Japan, Korea, Indonesia and Thailand with a total crude flour manufacture more than 25,000 tones (Parry, 2010). China and Japan is the largest producer of konjac flour and account for 60 and 28%, respectively, of global manufacture. Konjac flour which has been reported as a laxative agent was konjac glucomannan that originated from Japan (*A. konjac*). Therefore, in the present research attempts were made to authenticate and validate the ethno-medicinal potentials of *A. konjac* antimicrobial activity which could be alternate for current synthetic antimicrobial agents. The plant was selected for screening of antimicrobial efficacy against selected eight bacterial strains. The results of antimicrobial activity of crude dicholormethane (DCM), ethyl acetate, chloroform and methanol were tested.

MATERIALS AND METHODS

Collection of plant material

A. konjac was collected in the month of April 2014 from tribal pockets of Mount Abu district Sirohi, Rajasthan, India (Figure 1). This plant was used by tribal's in their day to day lives to cure different ailments and commonly known as ajgarikand.

Identification

This sample were authenticated and was given identification number and submitted in Ethno-medicinal Herbarium, Centre with potentials of Excellence funded by DST, JECRC University, Jaipur, India. Further, voucher specimens of *A. konjac* was deposited at herbarium of University of Rajasthan, Jaipur, India and was verified by senior taxonomist of department and provided with accession no. RUBL211565.

Bacterial strains

Eight species of human pathogenic bacteria were obtained from Max Hospital Delhi in the month of October 2014. The colonies were authenticated with American Type Culture Collection (ATCC); bacterial species used for testing were *Staphylococcus aureus (ATCC- 2921), Klebsiella pneumoniae (ATCC 700603), Enterobacter cloacae (ATCC 13047), Escherichia coli (ATCC 25922), S. aureus (ATCC 25923), Proteus mirabilis (ATCC 12453), Enterobacter cloacae (ATCC- 13047), Enterococcus faecalis (ATCC 29212)* and *Streptococcus pneumoniae (ATCC 6305)* and were maintained on nutrient broth media.

Preparation of extracts

The tuber corms were washed well followed by exterior sterilization using 1% of sodium hypochlorite. The tubers were sliced into pieces, shade dried, and powered using an electric blender. Powdered crushed plant material (1 kg) of selected species was successively extracted with dicholormethane, ethyl acetate, chloroform, methanol and water. Extraction was done by Soxhlet apparatus using solvents in the increasing order of their polarity. Later, each of the homogenates was filtered and the residue was re-extracted twice for complete exhaustion, and the extracts were cooled individually. The obtained solvent extracts were concentrated using vacuum distillation process *in vitro* and re-dissolved in solvent, whenever screened for antimicrobial activity (Harborne, 1998).

Cultivation of test microorganisms

Bacteria was cultured on Nutrient agar medium (NAM). The medium was prepared using 20 g agar, 5 g peptone, 3 g beef extract and 3 g of NaCl in 1 L distilled water and sterilized at 15 lbs pressure and 121°C for 25 to 30 min. Agar test plates were prepared for pouring approximately 20 ml of NAM into the Petri dishes (10 mm) under aseptic laminar hood conditions. A peptone solution was prepared (by mixing 0.5% peptone) in distilled water, followed by autoclaving and the cultures were maintained on this medium by sub-culturing at regular interval with an incubation at 37°C for 24 to 48 h. For preparation of test plates, in bacteria, 10 ml of the respective medium was poured onto the Petri plates and used for screening.

Bactericidal assay

For bactericidal assay *in vitro* Disc diffusion method was adopted (Gould and Bowie, 1952), because of reproducibility and precision. The different test organisms were proceeded separately using a sterile swab over previously sterilized culture medium plates and the zone of inhibition was measured around sterilized dried discs of Whattman No.1 paper (6 mm in diameter), which contained three different concentration of respective solvent (A = 1 mg of test extract/disc; B = 5 mg of test extract/disc; C = 10 mg of test extract/ disc) and tetracycline as reference drug (standard disc) separately. Further, treated discs were air dried at room temperature to remove any solvent, which may interfere with the strength, contaminations and inoculation. Initially the plates were exposed to low temperature for 1 h so as to allow the maximum diffusion of the compounds from the test disc into the agar plate and later, incubated at 37°C for 24 h in case of bacteria, after which the zone of inhibition could be made easily and calculated. Five replicates of each test extract were performed and the mean values were then referred. The zone of inhibition (IZ) in each result was recorded and the activity index (AI) was calculated respective to thestandard reference drugs (AI = Zone of inhibition of test sample / zone of inhibition of standard).

RESULTS AND DISCUSSION

A. konjac is known for its snake like stripes on stem and make its appearance unique in flora of Rajasthan. In Mount Abu the plant is grown in wild and known with some toxic principles. The results of antimicrobial activity of crude dicholormethane, ethyl acetate, chloroform and methanol are shown in Table 1. All the solvent extracts of Ajgarikand inhibited the growth of all the eight selected bacterial strains in a dose dependent manner. *Amorphophallus commutatus* was studied for morphological, phytochemical, and antibacterial properties (Krishna et al., 2013; Damle and Kotian, 2015), but till now systemic studies on the antimicrobial efficacy of *A. konjac* has not been studied so far. National Institute of Nutrition Standards recommended that *A. commutatus* possess all the edible part including macro and micro elements in adequate quantity (ICMR, 2009). Such studies provide a background for substitutes in ayurveda and provide a base for search of alternative plants from same family as nutraceuticals (Damle and Kotian, 2015; Pandey and Gupta, 2013). Therefore, to justify the ethno-medicinal use of *A. konjac* in ancient science, this scientifically validated the use.

Figure 2 shows various results of antimicrobial activity of crude dichloromethane (DCM), ethyl acetate, chloroform and methanol extracts. The results show that all the extract express appreciable efficacy against all the selected microorganisms. DCM extract C (10 mg/disc) possess maximum efficacy against *S. pneumoniae (*Inhibition zone = 20 mm; Activity Index = 1.25). The results show that efficacy of DCM extract are more than standard tetracycline. *S. pneumoniae* resides in nasopharynx of healthy carriers and cause community acquired pneumonia and meningitis resulted into coughing, sneezing etc. Therefore, use of DCM extract and bioactivity guided fractionation will lead to novel bioactives which will work as future antibiotics. Ethyl acetate extract also showed appreciable efficacy against the entire test organisms except *Proteus mirabilis* so using such extract in future can be used to isolate new chemical entity as broad spectrum drugs. It possess maximum efficacy against *S. aureus* (IZ = 18 mm; AI = 1.05) and *S. pneumoniae* (IZ = 25 mm; AI = 1.56). It is also noteworthy that the minimum inhibition concentration of both the ethyl acetate disc also possess efficacy

Table 1. Antibacterial activity of dichloromethane, ethyl acetate, chloroform and methanol extracts of *A. konjac*.

Solvent type	Extract		EC	SA	KP	SAB	PM	ECL	EF	SP
Standard (Tetracycline)		IZ	17	17	19	19	11	18	15	16
Dicholoro-methane	A	IZ	-	-	-	6	-	6	5	16
		AI	-	-	-	0.31	-	0.33	0.33	1
	B	IZ	-	5	-	7	-	6	6	13
		AI	-	0.29	-	0.36	-	0.33	0.4	0.81
	C	IZ	6	6	-	8	-	7	7	20
		AI	0.35	0.35	-	0.42	-	0.38	0.46	1.25
Ethyl acetate	A	IZ	5	16	5	6	-	8	6	23
		AI	0.29	0.94	0.26	0.31	-	0.44	0.4	1.43
	B	IZ	6	17	9	8	-	10	7	24
		AI	0.35	1	0.47	0.42	-	0.55	0.46	1.5
	C	IZ	7	18	11	6	-	10	8	25
		AI	0.41	1.05	0.57	0.31		0.55	0.53	1.56
Chloroform	A	IZ	6	-	8	6	-	6	5	6
		AI	0.35	-	0.42	0.31		0.33	0.33	0.37
	B	IZ	7	6	9	7	-	7	9	11
		AI	0.41	0.35	0.47	0.36		0.38	0.6	0.68
	C	IZ	9	8	9	8	-	8	8	9
		AI	0.52	0.47	0.47	0.42		0.44	0.53	0.56
Methanol	A	IZ	-	-	-	-	-	6	5	10
		AI	-	-	-	-	-	0.33	0.33	0.62
	B	IZ	-	6	-	6	6	6	6	-
		AI	-	0.35	-	0.31	0.54	0.33	0.4	-
	C	IZ	7	7	-	7	6	9	8	12
		AI	0.41	0.41	-	0.36	0.54	0.5	0.53	0.75

I.Z. = Inhibition zone showed by extract against microorganism in mm; AI = Activity index of extract; SA =*Staphylococcus aureus* (ATCC- 2921); KP = *Klebsiella pneumoniae* (ATCC 700603); EC = *Escherichia coli* (ATCC 25922); SAB = *Staphylococcus aureus* (ATCC 25923); PM = *Proteus mirabilis* (ATCC 12453); ECL = *Enterobacter cloacae* (ATCC- 13047); EF = *Enterococcus faecalis* (ATCC 29212); SP = *Streptococcus pneumonia* (ATCC 6305); - = no inhibition zone; A = 1 mg/disc; B = 5 mg/disc; C = 10 mg/disc.

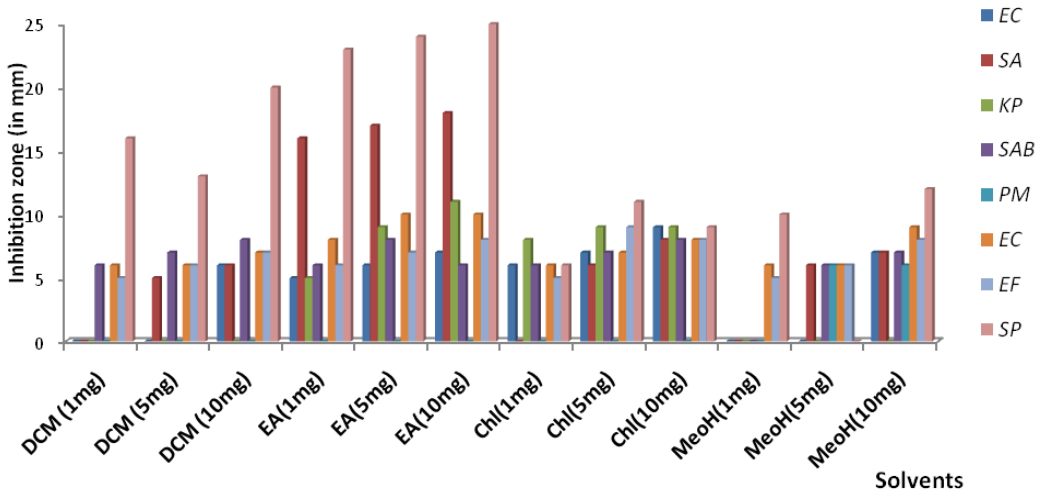

Figure 2. Antibacterial activity of dichloromethane, ethyl acetate, chloroform and methanol extracts of *A. konjac*. SA =*Staphylococcus aureus* (ATCC- 2921); KP = *Klebsiella pneumoniae* (ATCC 700603); EC = *Escherichia coli* (ATCC 25922); SAB = *Staphylococcus aureus* (ATCC 25923); PM = *Proteus mirabilis* (ATCC 12453); ECL = *Enterobacter cloacae* (ATCC- 13047); EF = *Enterococcus faecalis* (ATCC 29212); SP = *Streptococcus pneumonia* (ATCC 6305).

more than standard which is really a remarkable feature.

Further, processing of this extract will lead to generation of new antibiotics. Chloroform and methanol extracts showed significant efficacy against all the test microorganisms whereas the maximum efficacy against *S. pneumoniae* (IZ = 11 mm; AI = 0.68) and (IZ = 12 mm; AI = 0.75), respectively. The main cause of community acquired pneumonia and septicemia in HIV infected patients is caused by *S. pneumoniae*. Further, bioactivity guided fractionation from DCM extract of *A. konjac* can lead to isolation of pure compounds as novel antibiotic in future. Therefore, the extract can also be used for isolation of volatiles compounds with potentials so that the extract/ active fraction/ pure compounds can be used as nasal spray in future therapeutics.

Conflict of interests

The authors did not declare any conflict of interest.

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