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RESEARCH ARTICLE

Antibacterial property of *Aconitum heterophyllum* root alkaloid

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Abstract

In our earlier study, alkaloid extract from *Aconitum nagarum* root was reported for antibacterial activity, where aconitine was identified as bioactive compound. The aim of the current study was to analyze the antibacterial activity of the root alkaloid extract of another important *Aconitum* species, namely *Aconitum heterophyllum*. This alkaloid extract showed antibacterial activity against *S. aureus*, *B. bronchiseptica*, *B. subtilis*, *P. putida* and *X. campestris*, while this activity was negligible against *S. typhimurium*, *E. coli* and *P. fluorescence*. The extract resolved to 9 bands and none of the band corresponded to aconitine in thin layer chromatography (TLC). These bands were eluted and analyzed for antibacterial activity. Most of the eluted bands displayed antibacterial activity, which was comparatively high with the fifth (R_f: 0.47) and ninth (R_f: 0.91) band. Mode of action of these eluted bands was cidal for *S. aureus*, *B. bronchiseptica* and *B. subtilis*, while the effect was static for other bacterial species. Thus, the present study revealed the antibacterial activity of *A. heterophyllum* alkaloids from root was due to synergistic effect of different alkaloids.

Introduction

Aconitum heterophyllum Wall commonly known as 'Atis' belongs to Ranunculaceae family. It is a perennial herb which is found throughout the world and cultivated in tropic fields. It has been used in the treatment of various ailments such as hysteria, throat infection, dyspepsia, abdominal pain, diabetes and considered as a valuable febrifuge nervine tonic especially combating debility after malaria and hemoplegia (Dar et al., 2001). The plant has also been reported to possess antifungal, antiviral and immune-stimulant properties (Atal et al., 1986; Anwar et al., 2003; Pandey et al., 2004). Several substances having potential biological significance have been reported from *A. heterophyllum*, like benzoylmesaconine, mesaconitine, hypaconitine, heteratisine, heterophyllisine, heterophylline, heterophyllidine, atidine, isotisine, hetidine, hetisinone and benzoylheteratisine (Zhaohong et al., 2006). Other compounds such as flavonoids, tannins, saponins and sugars have also been isolated from *A. heterophyllum* (Pelliter et al., 1968).

Antibacterial activity of alkaloid extracts from the root of two *Aconitum* species (viz. *A. nagarum* and *A. elwesii*) from Manipur, India has also been reported by Sinam et al., 2011, 2012, 2013 revealing its medicinal importance. Further, aconitine was characterized as bioactive compound responsible for this activity of the alkaloid from *A. nagarum* root (Sinam et al., 2012). Considering the traditional uses and potential medicinal properties of *A. heterophyllum*, the current study was undertaken to investigate antibacterial activity of the root alkaloid extract from this plant.

Materials and methods

Alkaloid extraction

Aconitum heterophyllum roots were procured from a local ayurvedic herbal shop in Mumbai, Maharashtra, India. Alkaloids from these roots were extracted by the methods described by Ohta et al., (1997). Briefly, finely powdered roots were homogenized with 1M HCl in the ratio of 1:10 (w/v) in a mortar and pestle and filtered through double-layered cheesecloth.

The filtrate was centrifuged at 12,500 g for 20 min at ambient temperature (26 ± 2°C) and the supernatant was adjusted to pH 10 with ammonia solution (25%). The resulting suspension was extracted thrice with equal volumes of chloroform. The organic layer was pooled together and washed thoroughly with distilled water to remove ammonia. The organic phase was dried over anhydrous sodium sulfate to remove traces of water molecules and later evaporated with N_2 gas.

Detection of alkaloid

Mayer's reagent (K_2HgI_4) was prepared by dissolving mercuric chloride (1.36 g) and potassium iodide (5 g) in 100 ml of milli Q water. It was used for the qualitative detection of alkaloid in the herbal extracts (Wangchuk, 2004). One ml of extract was transferred to a petri dish and Mayer's reagent was added. The presence of alkaloid resulted in milky appearance due to the formation of alkaloid salt precipitate (Wangchuk, 2004).

Thin layer chromatography (TLC) of alkaloid extract

Silica-gel 60 F_{254} plates with aluminium backing (0.2 mm thickness) supplied by Merck were used for analytical TLC. For preparative TLC, plates were prepared with Silica Gel-G (E- Merck) Mumbai, India. Separation of alkaloid was carried out using diethyl ether - ethyl acetate in ratio 20:1 saturated with conc. ammonia as developing solvent system (Ohta et al., 1997). The resolved alkaloids were visualized under UV lamp (Camag, Switzerland) at 254 nm. All the resolved bands were scrapped separately, dissolved in methanol, centrifuged (15000g), supernatant was collected, dried using N_2 gas and dissolved in dimethyl sulfoxide (DMSO).

Analysis of antibacterial activity

Antibacterial activity of the crude alkaloid extract from the root and preparative TLC resolved bands were tested against different bacterial species including certain human pathogens, *Staphylococcus aureus, Salmonella typhimurium* (MTCC 98), *Bordetella bronchiseptica* (NCIM 2267), *Escherichia coli* (MG 1655), *Bacillus subtilis* (NCIM 2063), *Pseudomonas putida* (NCIM 2847), *Pseudomonas fluorescence* (NCIM 2059) and *Xanthomonas campestris* using disc diffusion method (An et al., 2004). In brief, the test bacterium was grown overnight in 10 ml of Luria Bertani broth (LB). The incubation temperature was 37°C for pathogenic bacteria including *S. aureus, S. typhimurium, B. bronchiseptica* and *E. coli* and 28°C for other microbes. Cultures were diluted to ~10^5 to 10^6 cfu / ml. 100 µl of this dilution was spread plated on a Luria-Bertani agar (LA) plate and the plates were dried under laminar air flow for 20 min. The alkaloid extract from root was tested at four different concentrations (12.5, 25, 50 or 100 µg / disc), whereas eluted TLC bands were analyzed at 50 and 100 µg / disc. DMSO without any extract served as a solvent control. Sterile paper discs (6 mm in dia.) were placed aseptically on the inoculated plate. 20 µl aliquot of the sample was transferred on the disc and the plates were later incubated overnight at appropriate incubation temperature as mentioned above. The diameter of the zone of inhibition was measured in mm.

Analysis of mode of antibacterial activity

Mode of antibacterial activity, whether bactericidal or bacteriostatic, was determined as per our previous report (Sinam et al., 2013). Briefly, twenty random points from the zone of inhibition were re-spotted on a fresh LA plate and observed for the growth of any viable colony. No growth from the spot was indicative of bactericidal action, whereas growth from the spot indicated bacteriostatic action. The experiment was repeated at least twice to confirm the observations.

Results

Alkaloid detection and TLC analysis

In a qualitative assay, the alkaloid extracts from *A. heterophyllum* turned milky upon addition of Mayer's reagent which confirmed the presence of alkaloid in the samples. During TLC analysis, crude alkaloid extract resolved into 9 distinct bands. There was no spot that corresponded to the standard aconitine (R_f: 0.73) (Fig. 1a & b).

Antibacterial activity and possible mode of action of alkaloid extract

The antibacterial activity of the alkaloid extract from the root of *A. heterophyllum* as well as eluted TLC bands was tested against different bacteria including certain human pathogens. For a comparative characterization, the antibacterial activity was categorized as weak, moderate and strong depending upon zones of inhibition (weak: 7-8 mm, moderate: 9-11 mm and strong: ≥12 mm).

The crude alkaloid extract displayed moderate to strong level of antibacterial activity against *S. aureus, B. bronchiseptica, B. subtilis, P. putida* and *X. campestris* at higher concentration of 100 µg/ disc (Table 1). This activity was almost absent against *S. typhimurium, E. coli* and *P. fluorescence* at the same concentration (Table 1). The mode of antibacterial activity (whether bactericidal or bacteriostatic) was also determined for these crude and TLC eluted alkaloid fraction. The alkaloid extracts showed bactericidal effect against *S. aureus, B. bronchiseptica* and *B. subtilis*, whereas for other bacterial species the effect was bacteriostatic even at the concentration of 100 µg/ disc (Table 3). MIC value of the crude alkaloid extract ranged from 94 (*B. bronchiseptica*) to 500 (*P. fluorescence*) (Table 1). Further, similar results were observed with the 9 eluted preparative TLC bands (Table 2). However, among these bands, 5^{th} (R_f: 0.47) and 9^{th} (R_f: 0.91) band displayed comparatively higher activity (Table 2). Figure 2 depicts antibacterial activity of the TLC eluted 5^{th} band against some of the bacterial species. All

the eluted bands showed bactericidal effect against *S. aureus, B. bronchiseptica* and *B. subtilis*, whereas bacteriostatic effect was observed against *P. putida* and *X. campestris* (Table 3).

Table 1
Antibacterial activity, mode of action and MIC value of alkaloids extract of *Aconitum heterophyllum* root.

Bacteria	Zone of inhibition (mm) at µg/disc				Mode of action	MIC (µg /ml)
	12.5	25	50	100		
Staphylococcus aureus	-	-	10	12	C	125
Salmonella typhimurium	-	-	-	-	-	-
Bordetella bronchiseptica	-	9	10	11	C	94
Escherichia coli	-	-	-	-	-	-
Bacillus subtilis	-	9	11	12	C	62.5
Pseudomonas putida	-	7	8	10	S	94
Pseudomonas fluorescence	-	-	-	7	S	500
Xanthomonas campestris	-	7	8	12	S	*

-: No activity; *: Not detremined; C:bactericidal; S:bacteristatic

Table 2
Antibacterial activity of preparative TLC isolated compounds from *A. heterophyllum* root

Band no	Concentrations (µg/disc)	Disc diffusion method (inhibition zone, mm)				
		S.a.	B.b.	B.s.	P.p	Xc
1	50	8	7	7	-	7
	100	11	10	10	8	8
2	50	-	7	8	-	-
	100	10	10	10	8	7
3	50	-	8	9	-	7
	100	8	10	10	8	10
4	50	-	7	8	-	-
	100	-	10	11	7	7
5	50	8	8	8	-	8
	100	12	11	11	7	10
6	50	8	8	7	-	7
	100	9	9	9	7	8
7	50	-	8	8	-	7
	100	9	11	10	8	9
8	50	-	8	9	-	7
	100	10	11	11	7	10
9	50	9	9	7	-	7
	100	12	11	8	-	9

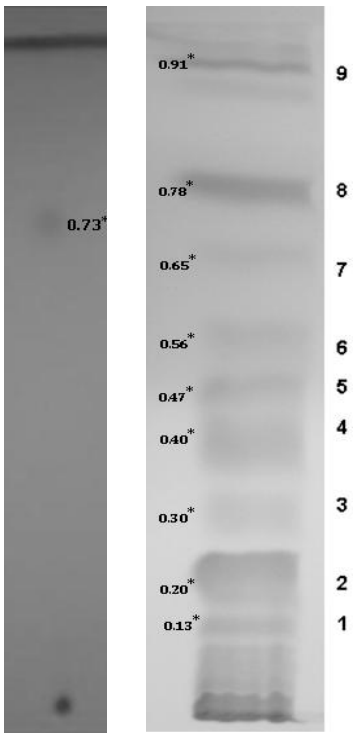
-, no activity; S.a, *Staphylococcus aureus*; B.d, *Bordetella bronchiseptica*; B.s, *Bacillus subtilis*; P.p, *Pseudomonas putida*; X.c, *Xanthomonas campestris*.

Table 3
Mode of antibacterial activity of alkaloid fractions of *A. heterophyllum* root obtained by preparative TLC

Band no.	Mode of antibacterial activity shown by bacteria				
	S. aureus	Bordetella	Bacillus	P. putida	Xcg
1	C	C	C	S	S
2	C	C	C	S	S
3	C	C	C	S	S
4	C	C	C	S	S
5	C	C	C	S	S
6	C	C	C	S	S
7	C	C	C	S	S
8	C	C	C	S	S
9	C	C	C	S	S

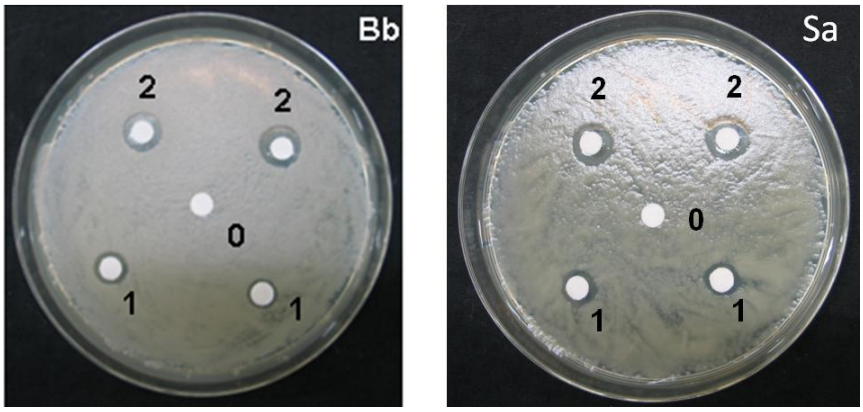
C- Cidal; S- Static

Fig. 1 TLC profile



1a) Standard aconitine
1b) *A. heterophyllum* root alkaloid
*- R_f value

Fig 2. Antibacterial activity shown by band 5 of the TLC plate against human pathogens



Bd, *Bordetella bronchiseptica*; Sa, *Staphylococcus aureus*; 0- Control; 1-50 µg; 2-100 µg

Discussion

The antimicrobial activity of alkaloid extract from natural sources have significance as pathogens may cause acute health problems (Smith and Secoy, 1981). *S. aureus* infection mainly results in atopic dermatitis and toxic shock syndrome (TSS). *S. typhimurium* causes typhoid and gastroenteritis, whereas *E. coli* infection results in diarrhea and dysentery-like syndrome in several cases. *B. bronchiseptica* infects the respiratory tracks of small mammals such as cats, dogs, rabbits and occasionally to humans.

The Gram-negative bacteria, *S. typhimurium*, *E. coli* and *P. fluorescence* were found to be comparatively less susceptible to the most of the alkaloid extract. All the Gram-positive bacteria, *S. aureus* and *B. subtilis* tested were sensitive to the alkaloid extract. Similarly, total alkaloids extract from *Mitragyna inermis* have been reported for comparatively higher antibacterial activity against Gram-positive bacteria (Zongo et al., 2009). A higher resistance in the Gram-negative bacteria may be due to the presence of outer membrane, which acts as barrier to the penetration of several antibiotics, and the enzymes present in the periplasmic space, which degrade exogenous molecules (Angenot et al., 1991; Tanaka et al., 2006). Also, similar to our findings, alkaloid extract from bark of *Holarrhena pubescens* was reported for bactericidal activity against *S. aureus* and *B. subtilis* (Chakraborty and Brantner, 1999).

Some alkaloids including aconitine from *Aconitum* species are known to cause toxic effects at higher concentrations (Bisset, 1981). The presence of broader activity spectrum alkaloids in plants may be meant for their multipurpose defense (Wink et al., 1998). In the current study with *A. heterophyllum* alkaloids from root, unlike our earlier study with *A. nagarum*, aconitine was not observed. This could be one possible reason for comparatively more usage of this *Aconitum* species as ethnomedicine. The antibacterial activity of alkaloid extract from this plant was found to be due to synergistic effects of several alkaloids. Although the mechanism for antibacterial activity of alkaloids is not yet known, some are reported to inhibit DNA synthesis through topoisomerase inhibition and DNA intercalation (Karoul et al., 2005). So further studies on these alkaloids will shed some light on the mechanism of action against these pathogenic bacteria.

Conclusion

The study established the antibacterial property of alkaloid extracts of *A. heterophyllum* root against different bacteria including human pathogens. Antibacterial activity of root alkaloid extract was due to synergistic effect of alkaloids. The root alkaloids from this *Aconitum* species thus provide an alternate ethnomedicine having wider spectrum of antibacterial activity.

References

1. **An BJ, Son JH, Kwak JH, Park JM, Lee JY, Jo C, Byun MW** (2004). Biological and antimicrobial activity of irradiated green tea polyphenols. Food Chemistry 88:447-451.
2. **Angenot L, Quentin-Leclercq J, Phillipson DJ, Warhurst DC, O'Neill MJ, Bray DH, Wright CW** (1991). Antiamoebic and antiplasmodial activities of alkaloids isolated from *Strychnos usambarensis*. Planta Medica 57:337-340.
3. **Anwar S, Ahmad B, Sultan M, Gul W, Islam N** (2003). Biological and pharmacological properties of *Aconitum chasmanthum*. J. Biol. Sci.; 3:989-993.
4. **Atal CK, Sharma ML, Koul A, Khajuria A** (1986). Immunomodulating agents of plant origin. I: Preliminary screening. Journal of Ethnopharmacology; 18: 133-141.
5. **Bisset NG** (1981). Arrow poisons in China. Part II. *Aconitum*-Botany, Chemistry, and Pharmacology. Journal of Ethnopharmacology; 4:247-336.
6. **Chakraborty A and Brantner AH** (1999). Antibacterial steroid alkaloids from the stem bark of *Holarrhena pubescens*. Journal of Ethnopharmacology 68:339-344.
7. **Dar GH, Bhagat RC, Khan MA** (2001). Biodiversity of Kashmir Himalaya. India: Valley Book House. p. 120-176.
8. **Karoul D, Savadogo1 A, Canini A, Yameogo1 S, Montesano C, Simpore J, Colizzi V,Traore AS** (2005). Antibacterial activity of alkaloids from *Sida acuta*. African Journal of Biotechnology 4:1452-1457.
9. **Ohta H, Seto Y, Tsunoda N** (1997). Determination of aconitum alkaloids in blood and urine Samples. I. High-performance liquid chromatographic separation, solid-phase extraction and mass spectrometric confirmation. Journal of Chromatography B 691:351-356.
10. **Pandey H, Nandi SK, Kumar A, Palni UT, Chandra B, Palni L** (2004). In vitro propagation of *Aconitum balfourii* Stapf: an important aconite of the Himalayan alpines. Journal of Horticultural Science and Biotechnology, 79: 34-41.
11. **Pelliter SW, Aneja R, Gopinath KW** (1968). The alkaloids of *Aconitum heterophyllum* wall: Isolation and characterization. Phytochemistry, 7:625-635.
12. **Sinam YM and Shantibala GA.** (2011). Seasonal variation of Bioactive alkaloids of *Aconitum* spp. In Manipur, India. The Bioscan. 6(3):439-442.

13. **Sinam YM, Sanjeev K, Sachin H, Satyendra G, Chatterjee S, Prasad S.V, Shantibala GA, Arun S.** (2012). Isolation and identification of a major antibacterial compound from Indo-Himalayan *Aconitum nagarum.* Asian Pacific Journal of Tropical Diseases. 2(2): S878-S882.

14. **Sinam YM, Sanjeev K, Sachin H, Satyendra G, Shantibala GA, Arun S.** (2013). Morpho-phenological and antibacterial characteristics of *Aconitum* spp. Notulae Scientia Biologicae. 5(2):189-197.

15. **Smith AE and Secoy DM** (1981). Plants used for agricultural pests control in Western Europe before 1850. Chemistry and Industry 12-17.

16. **Tanaka JC, da Silva AC, de Oliveira AJ, Nakamura CV, Filho BP** (2006). Antibacterial activity of indole alkaloid from *Aspidosperma ramiflorum***.** Brazilian Journal of Medical and Biological Research 39:387-391.

17. **Wangchuk P** (2004). Bioactive alkaloids from Medicinal Plants of Bhutan. Thesis, Master of Science University of Wollongong, Australia.

18. **Wink M, Schmeller T, Latz-Brüning B** (1998). Modes of action of allelochemical alkaloids: interaction with neuroreceptors, DNA, and other molecular targets. Journal of Chemical Ecology 24:1881-1937.

19. **Zhaohong W, Wen J, Xing J, He Y** (2006). Quantitative determination of diterpenoid alkaloids in four species of *Aconitum* by high performance liquid chromatography (HPLC). J. Pharmaceut. Biomedical. Anal., 40:1031-1034.

20. **Zongo CE, Akomo O, Savadogo A, Obamc LC, Koudou Traore JA** (2009). *In vitro* antibacterial properties of total alkaloid from *Mitragyna inermis* (Willd.) O. Kuntze, a West African traditional medicinal plant. Asian Journal of Plant Sciences 8:172-177.