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Biological and Pharmacological Properties of *Aconitum chasmanthum*

[1]Saeed Anwar, [2]Bashir Ahmad, [1]Muhammad Subhan, [1]Waseem Gul and [2]Nazar-ul-Islam
[1]Chemistry Department, Islamia College, Peshawar, Pakistan
[2]Department of Pharmacy, University of Peshawar. Peshawar, Pakistan

Abstract: Crude methanolic (90%) extract of ***Aconitum chasmanthum*** as well as various fractions of the same obtained by using different solvent systems were screened for biological and pharmacological activities such as antifungal, antibacterial, insecticidal and Brine shrimp cytotoxic activities. It was observed that the antifungal activity varies from negligible to strong depending upon the solvent system used for the extraction. All the fractions exhibited remarkable antifungal activity against ***Trichophyton mentagrophyte***. However the activity against other organisms varies from no activity to strong inhibition. Antibacterial, insecticidal activities and Brine shrimp cytotoxicity results are also presented.

Key words: *Aconitum chasmanthum*, biological and pharmacological activities

Introduction

The Genus ***Aconitum*** has had fearsome reputation since antiquity. In ancient Greek mythology, ***Aconitum*** were said to have born of the saliva of Cerberus, guard dog to Hell (Tripp, 1970; Anonymous 1958 and Le Strange, 1977) and has been suggested that genus ***Aconitum*** takes its name from the ancient Greek for Javelin ακοτιον reflecting the use of plants in poisoning such weapons. Certainly various species have been used for this purpose, as well as in the preparation of poisonous baits for animals (hence the old English name wolfsbane for the genus). There is also a history of the use of the plant ***Aconitum*** in aphrodisiacs (Gunther, 1959) and in potions used by witches (Le Strange, 1977 and Emboden, 1979). John Gerard, the Elizabethan herbalist provided one of the best descriptions of the effects of consuming the plant in his ***Herball***: "Their lips and tongs swell forthwith, their eyes hang out, their thighs are stiff and their wits are taken out of them" (John, 1992). The plant *A. **chasmanthum*** stapf. ex. Holmes (Ranunculaceae), a plant indigenous to the western Himalayas, is a robust perennial plant, reaching 2-4 ft in height and growing from a tuberous root-stock. It is abundant in alpine zones, between altitude of 4000 and 22,00 m, from Chitral to Hazara and Kashmir (Chopra ***et al.***, 1949). All parts of the plant have been recognized to be dangerously poisonous for humans, sheep and goats (Ibid). Previous work had shown the toxins to be alkaloidal in nature of which a few were identified.

The main objective of our work was to explore the alkaloid composition of the plant in more detail and to see if other bases were present which might contribute to the total toxic properties and by doing so aid in the design of a rational treatment for the poisoning, However, before taking up the main task of isolation, purification and identification/ characterization of

pure alkaloids, it was essential to subject the crude extracts and various fractions of the plant to screening for any biological/pharmacological activities.

Materials and Methods

Plant materials: The plants of *Aconitum chasmanthum* stapf. ex. Holmes were collected in August 1995 from Resquta top in Azad Jammu and Kashmir. It was identified by Saood Omar of the Botany Department, University of Karachi and a voucher specimen # 66807 was deposited in the Herbarium of the Botany Department, University of Karachi, Pakistan.

Extraction

A large quantity of the fresh plant collected from the Resquta top in Kashmir, was air dried, crushed; and was subjected to the extraction procedures as described in Fig. 1.

The crude methanolic extract and all the other fractions were concentrated under vacuum at low temperature (30°C) in order to avoid any thermal decomposition.

Antifungal Activity

The Agar Diffusion Method (Atta-ur-Rahman *et al.*, 1991) Griseofulvin was used as the standard drug determined The antifungal activity of the plant. The crude extract was dissolved in DMSO

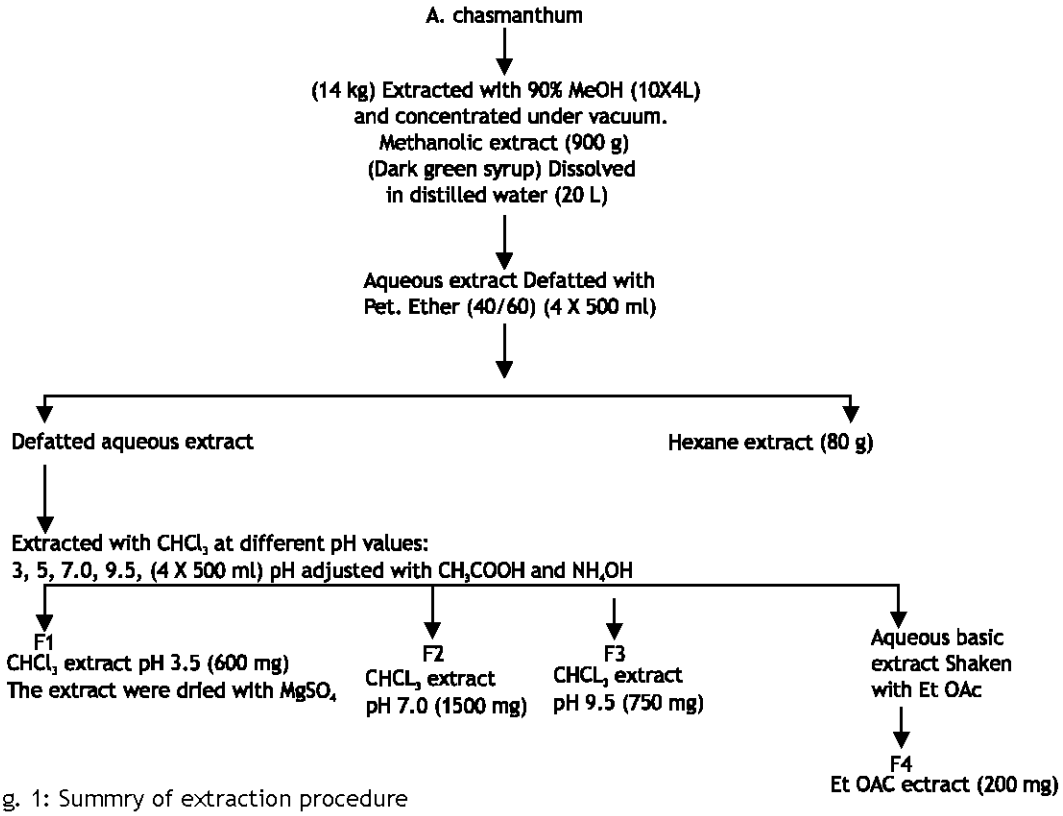

Fig. 1: Summry of extraction procedure

(50 mg 5^{-1} ml). Sterile SDA (Sabouraud's dextrose agar) medium (5 ml) was placed in a test tube and inoculated with the sample solution (400 µg ml^{-1}) kept in slanting position, at room temperature, overnight. The fungal culture was then inoculated on the slant. The samples were incubated for 7 days at 29°C and growth inhibition was observed.

Antibacterial Activity

The antibacterial activity was determined by the Agar Well Diffusion Method (Atta-ur-Rahman, ***et al.***, 1991). In this method 1 loop full of 24 h old culture containing approximately 10^4-10^6 CFU was spread on the surface of Mueller-Hinton Agar plates. Wells were dug in the medium with the help of a sterile metallic cork borer. Stock solution of the test sample in the concentration of 1mg ml^{-1} were prepared in dimethyl sulfoxide (DMSO) and 100 µl and 200 µl of each dilution was added in their respective wells. Control well received only 100 µg $µl^{-1}$ and 200 µg $µl^{-1}$ of DMSO. The antibacterial activity of crude extract was compared with three standard antibacterial antibiotics, ampicillin, amoxicillin and cefuroxime.

Brine Shrimp Lethality Test

Brine Shrimp bioassay is an excellent method of determining cytotoxicity of crude plant extracts and pure natural products (Meyer ***et al.***, 1982). In this method, crude samples were tested at initial concentration of 10, 100 and 1000 µg ml^{-1} in vials containing 5 ml of brine solution and ten shrimps in each of the three replicates. After 24 h, the shrimps that survived in this medium were counted. A simple computer programme (Finney Programme) to estimate the LD50 value of the extract processed the data obtained.

Insecticidal Activity

Insecticidal activity was determined by contact toxicity method. In this method an artificial diet was prepared having composition of wheat bran (4 g), yeast (40 g), agar (4g) and distilled water (1L). The crude plant extract was dissolved in MeOH (1:1) and diluted to different concentrations, like 500 ppm, 50 ppm and 5ppm. This solution was taken in 10 ml vial to make a thin film lined with the wall. The solvent was evaporated and a measured amount of the prepared diet was placed in this vial. The third instar larvae of ***Drosophila melanogaster*** (fruit fly) was transferred carefully with cotton plug on the base of vial and incubated at 27°C for a period of 6-12 days. After incubation, the percent mortality was observed.

Results and Discussion:

Different fractions of *A. **chasmanthum*** obtained by extraction with different solvent systems were tested against human, animal and plant pathogens and the results are given in the Table 1. It was observed that in case of ***Trichophyton mentagrophyte*** all the fractions exhibited strong antifungal activity. Whereas for the rest of the pathogens, the antifungal activity is quite diversified, ranging from no activity to strong inhibition. The most significant antifungal activity for the majority of pathogens was shown by the ethyl acetate fraction of *A. **chasmanthum***.

Table 1: Antifungal activity of various fractions of *Aconitum chasmanthum*

Name of Organism	Acidic Chloroform (pH 3.5) fraction	Neutral Chloroform (pH 7) fraction	Basic Chloroform (pH 9.5) fraction	Ethyl Acetate fraction
Allescheria boydii	+	+	-	-
Aspergillus niger	-	+	+	++
Candida albicans	-	-	-	-
Curvularia Lunata	-	-	-	-
Drechslera rostrata	+	-	-	+++
Microsporum canis	-	++	++	-
Nigrospora oryzae	+	+	+	+++
Pleurotus ostreatus	-	-	-	+++
Stachybotrys atra	++	-	+++	+++
Trichophyton mentagrophyte	+++	+++	+++	+++
Trichophyton longifusus	+	-	+	+
Trichophyton semii	+++	+++	+	+

+++ (strong inhibition), ++ (Medium Inhibition), + (weak inhibition), - (no activity)

Table 2: Antibacterial activity of crude methanolic extract against gram positive and gram negative microorganisms

		Zone of Inhibition (mm)		Standard Drugs					
				Ampicillin		Amoxicillin		Cefuroxime	
		100 µG	200 µg						
Group	Bacterial Culture	100 µl^{-1}	100 µl^{-1}	100 µg	200 µg	100 µg	200 µg	100 µg	200µg
Gram Positive	*Staphylococcus aureus*	--	--	16	17	17	18	16	17
	Streptococcus pyrogenes	--	--	17	19	16	18	16	18
	Corynebacterium agalactiae			14	16	14	16	14	15
	Corynebacterium diphtherie	--	--	10	12	10	12	11	13
	Bacillus Subtilus	--	--	16	18	16	18	10	17
Gram Negative	*Escherichia Coli*	--	6	18	19	17	18	17	18
	Salmonella Typhi	--	--	14	15	14	15	13	14
	Shigella Boydii	--	6	10	13	13	14	14	15
	Klebsiella Pneumonia	--	6	10	12	11	14	11	14
	Proteus mirabilis	--	6	17	18	14	16	16	18
	Pseudomonas aeruginosa	--	--	--	--	--	--	--	--

Table 3: Insecticidal bioassay for crude methanolic extract of *A. Chasmanthum*

Concentration in (ppm)	% Mortality	
	Test	Standard
500	100%	+100%
50	30	+100%
05	Zero	+100%

Observed :LD_{50} = 71000 µg ml^{-1}, G= - upper = Nil, Lower, Nil, Standard insecticide used = Atropine
Insect placed/single Vial = 5 larvae, Incubation period = 48 h

The antibacterial activity of crude methanolic extract was tested against gram positive and gram negative microorganism and the results are presented in Table 2. At low concentrations (100 µg), the activity for both types of microorganisms was nonexistent. However, at the highest

concentration (200 µg) the crude extract did show a weak inhibition of gram negative microorganisms. In comparison with standard inhibitors (Ampicillin, Amoxicillin and Cefuroxime), however, these results were negligible. Insecticidal bioassay for the crude methanolic extract was carried out and was compared with that of the standard insecticide (atropine). At the highest concentration (500 ppm) the activity was comparable to that of the standard (Table 3), whereas at low concentration (5 ppm), the activity went down to zero. The Brine Shrimp Lethality test when carried out with crude methanolic extract has shown that LD_{50} is >1000 µg ml^{-1}.

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