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ANTICANCER AGENTS OF *ARISAEMA JACQUEMONTII* BLUME

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The account of the physiological properties of the genus *Arisaema* is presented. The petroleum benzene, chloroform and methanol extracts of *Arisaema jacquemontii* Blume have been found to be active against cancer. The methanol extract of the plant has yielded a new compound, arisaeminone (1). Its structure has been assigned on the basis of spectral studies.

Key words.:*Arisaema jacquemontii*, Physiological properties, Cancer activity.

Introduction

The genus *Arisaema* belongs to the Araceae family. The 14 species of the genus are commonly found in the northern regions of Pakistan and in Kashmir [1]. The genus is reputed for its physiological properties in the folk medicine system.The root stock of *A. triphyllum* when cooked provided an Indian food. The red barries formed as the spadix are poisonous to humans but are eaten by many wild animals [2]. *A. amurense, A. consenquincum, A. heterophyllum* and *A. tatarinowii* are medicinal and find use for the relief of various ailments. Their tubers are bitter in taste, hot, poisonous and are used as internal medicine in cases of apoplexy, blood poisoning and convulsions of children [3]. Externally, tubers are used for poulticing swellings. The powdered corm is mixed with saliva and used as an antiseptic for itching follicles. The corm is also remedy for stomachache and the leaf is applied to swellings. The tubers are roots of *A. flavum* are also stated to have poisonous properties and are applied in powdered form to cure snake bite. The tubers are also used to kill worms. The fruit possess deleterious effects on mouth and causes irritation and swelling when chewed. The corm of *A. amurense* Maxin are used as anticonvulsant, expectorant, peptic and are good to prevent tremor [3]. The tubers of *A. japonicum* BI is used as a tonic, bechic, expectorant and a sedative for convulsions. The rhizome or tuber may be ground to powder and spread on cotton, then applied to swelling, used to treat numbness after a stroke, headache, wound, from falling and scrofula. The tubers of *A. ringens* Schott are used as having vermifuge, antidotal and bechic properties. The corm of *A. dilatatum* Buchet is used as an expectorant to relieve coughs [3]. The corm of *A. Lobatum* is poisonous, appplied as an antiseptic on malignant sores. *A. wilsonii* has mitogenic activity for human lymphocytes [4]. The urticating juice of the stems of *A. qarrethil* Gaynep is reputed to be toxic and used in arrow poison [5]. The roots of *A. speciosum* (the curious cobra lily) are antidote and tubers are given to sheeps as remedy for colic and to kill worms which infest cattle during the rainy season [6].

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In recent investigations it has been shown that various species of the genus *Arisaema* contain DNA damage inducing components, and the methyl ethyl ketone and methanol extracts of the plant showed anticancer activity in rad6 and rad52 yeast assays. It has also been found that the active components obtained from the fractionation of the active extracts of the plant are responsible for the different patterns (difference in their selectivity in rad 6 and rad 52 strains) of activity. Some investigations on the genus are reported [4-11] but no significant work has been done on the chemical constituents of *A. jacquemontii* Blume [11].

Arisaema jacquemontii Blume is widely distributed in northern parts of Pakistan and in Kashmir, found commonly in forest opening at the range 6000-12000 ft. above the sea level. The plant is locally known as " Surganda" and " Sap-ki-khumb" or "Sap-ki-booti" and is famous for its poisonous properties and also commonly known as dangerous plant. The latter name is given to the plant may be only due to its resemblance with the snake, cobra. The extracts of *A. jacquemontii* Blume have been found to be anticancer. The petroleum benzene, chloroform and methanol extracts of the plant have shown activity in rad6 and rad 52 yeast assays and found least active in rad+ (Table 1). The MeOH extract was subjected to dose response experiments which show much larger IC_{12} value in favour of rad 52. The two fold increase of IC_{12} value, in favour of rad 52 against rad+, show anticancer extract. As a result of investigations on the MeOH extract of *A. jacquemontii*, the isolation and structure determination of a new compound is reported here, arisaeminone [1]. Its structure has been elucidated on the basis of spectral studies.

Results and Discussion

The plant material was dried and extracted with petroleum benzene, chloroform, methanol and distilled water. Each of the extracts were subjected to bioassay experiments* using

* The bioassay experiments were conducted by Professor L. L. A. Gunatilaka with the courtesy of Professor David. G. I. Kingston at the Department of Chemistry, Virginia Polytechnic Institute and State University, Blacksburg, Virginia, USA. Their contribution is highly acknowledged.

TABLE 1.

Extract	Dose in ug/ml	Bioassy* zone of inhibition in rad52	IC^{+}_{12} rad52	IC^{+}_{12} rad+
Petroleum benzene	9000	12mm	-	-
Chloroform	7700	16mm	-	-
Methanol	8000	20mm	500	16000
Water (distilled)	8000	Not active	-	-

*Experiments performed in DMSO/MeOH(1:1); positive control for the strains are: rad 52, camptothecin at 5 mg/ml; rad+, camptothecin at 200 mg/ml. +Inhibition concentration causes 12 mm zone.

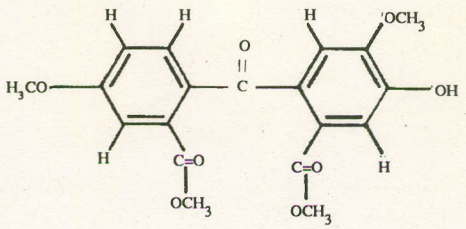
rad+ and rad52 strains. The bioassay results are summarized in Table 1. The IC_{12} values in case of methanol extracts is also calculated by the linear regression method. The petroleum benzene extract showed an inhibition zone of 12 mm at 9000 µg/ml dose in rad 52 yeast assay while the chloroform extract showed an inhibition zone of 16 mm at 7700 µg/ml dose. The methanol extract showed an inhibition zone of 20 mm at 8000 µg/ml dose in rad52 yeast assay. These experiments showed that the petroleum benzene, chloroform and methanol extracts are active against cancer while water extract showed no activity at all. These experiments also revealed that most of the activity is lying in the methanol extract. The dose response experiments were performed on the methanol extract and the IC_{12} values are calculated (Table 1). The methanol extract has an IC_{12} value of 500 and >16000 in rad52 and rad+ assays, respectively. An IC_{12} differential of 3 fold or larger in favour of the rad52 mutant versus the repair proficient wild type (rad+) indicates a lead extract. The MeOH extract was partitioned between BuOH and H_2O and the 2 fractions were bioassayed. The BuOH fraction showed an IC_{12} of 120 whereas the water fraction was inactive at 8000 µg/ml dose.

The MeOH extract was dried (3.4 g) and subjected to preparative thin layer chromatography (PTLC) with petroleum ether/chloroform (1:9) as the solvent system. This afforded a semi-pure compound which was further purified on PTLC plates (silica gel) with petroleum ether/chloroform (2:8) as the solvent system. This resulted a pure compound, arisaeminone (1) as an amorphous solid (R_f=0.68). It gave a blue colour reaction with molybdophosphoric acid reagent and pink colour reaction with anisaldehyde-sulphuric acid reagent.

The UV spectrum of the compound showed λ_{max} absorptions at 204 nm, (log ε= 4.22) 242nm (log ε = 3.75) 257 nm (log ε = 3.87) 278- (log ε = 3.86) and 350 nm (log ε = 3.77). The IR spectrum displayed an important absorption at 3500 cm^{-1} which indicated the presence of hydroxyl group in the molecule. Another strong band at 1645 cm^{-1} showed the presence of an α–β unsaturated carbonyl (C=C-CO-)function in the molecule.

The high resolution mass spectrum (recorded on Finnigan MAT- 312 mass spectrometer) displayed the molecular ion peak at *m/z* 374.1001, consistent with the molecular formula $C_{19}H_{18}O_8$ indicating 11 degrees of unsaturation in the molecule. Other prominent peaks were found to occur at *m/z* 359, 358, 346, 331, 316, 285, 273 and 155. The peaks at *m/z* 359, 346, 331 and 316 corresponded to the loss of methyl, CO, $COCH_3$ and $COOCH_3$-1 group from the molecular ion.

The ^{1}H-NMR spectrom ($CDCl_3$; 400 MHz) of the compound 1 revealed the presence of 18 protons in the molecule. The 5 proton resonances appeared in the aromatic region while 12 proton resonances (4 singlets, each integrated for 3 protons) appeared in the methoxy proton resonances region. A 3H singlet at δ 3.85 was assigned to the 5'-OCH_3 protons. Its upfield chemical shift may be attributed due to the sheilding influence of the 4'- OH group. A 3H singlet at δ 3.92 was assigned to the 4-OCH_3 protons. The two 3H singlets each at δ3.95 and δ3.98 were assigned to the 2 methyl protons of ester groups at 2- and 2' carbons respectively. The two singlets, each integrated for 1 proton, appeared at δ5.96 and δ6.49, were assigned to the 6'-H and 3'-H protons respectively. A doublet at δ 7.03 ($J_{\sigma,5}$ = 8.45 Hz) was assinged to the 6-H while a double doublet appeared at δ7.66 ($J_{5,\sigma}$ = 8.45 Hz, $J_{5,3}$ =2.01 Hz)was assigned to 5-H. The larger coupling constant ($J_{5,\sigma}$=8.45 Hz) indicating ortho coupling with the 6-H while the smaller coupling constant ($J_{5,3}$ = 2.01 Hz) observed due to meta coupling with the 3-H. The 3-H appeared at δ 7.70 ($J_{3,5}$ = 2.01Hz) as a doublet. A singlet at δ 12.59 was assigned to the phenolic 4'-OH proton [12]. On the basis of the above spectral studies we have assigned Structure 1 to arisaeminone.



Structure 1

Experimental

Plant material. The plant meterial was collected in Aug., 1990 from Murree, 54 km from Islamabad. The plant was identified by the plant Taxonomist, at the Department of Botany, University of Azad Jammu and Kashimir, Muzaffarabad.

*Extraction procedure**. The plant was air-dried (50 g) and extracted with petroleum benzene (300 ml; 1st. extraction) at room temperature (30-35°). The petroleum benzene was removed by filtration and dried (0.5 g). The residue was dried

* The plant material was allowed dip in the solvent for at least 4 days for each of the above extractions and the plant material was dried after each extraction and before the introduction of the next solvent.

and extracted with chloroform (300 ml; 2nd. extraction). The $CHCl_3$ extract was filtered off and dried (0.7 g). The plant material was dried and then extracted with MeOH (350 ml; 3rd. extraction). The MeOH was removed by filtration, which afforded a light-yellow coloured material (3.4 g). The fourth extraction was carried out in distilled water. This extract was also concentrated which afforded light yellow coloured material. Each of the extracts were subjected to bioassay experiments in rad+(RS188N) and rad52 (RS322N) yeast assays. The result are summarised in Table 1.

Isolation of arisaeminone (1). The methanol extract (3.4 g) of *Arisaema jacquemontii* was dired and subjected to the PTLC experiments silica-gel (GF-254) precoated plates with petroleum ether/chloroform (1.0:9.0) as the solvent system. This afforded a semi-pure compound which was further purified on silica-gel plates with petroleum ether/ chloroform (2.0:8.0) as the solvent system. This afforded a pure compound, arisaeminone 1 (R_f=0.68) as an amorphous material which gave blue colour reaction with molybdophosphoric acid reagent and pink colour reaction with anisaldehyde reagent.

UV (MeOH) λ_{max} nm (long ε): 204 (4.22), 242 (3.75), 257(3.87) 278(3.86) and 350(3.97); IR ($CHCl_3$ υ_{max}, cm^{-1} (function); 3500 (O- H), 2920(C-H), 1590(C=C), 1645(C=O) and 1000(C-O); MS (*m/z*); 374 (100%), 359, 358, 346, 331, 316, 285, 273, 231, 181, 155, 142, 135, and 121; ^{1}H-NMR ($CDCl_3$, 400 MHz)δ : 3.85 (3H, s, 4'-OCH_3, 3.92 (3H,s, 4-OCH_3), 3.95 (3H, s, 2-$COOCH_3$) 3.98 (3H, s, 2'-$COOCH_3$) 5.96 (1H, s, 5'-H), 6.49(1H, s, 3'-H), 7.66 (1H, dd, J 5, σ = 8.45Hz, $J_{5,3}$=2.01 Hz, 5- H), 7.03 (1H, d, Jσ, 5=8.45 Hz, 6-H), 7.70 (1H, d, $J_{3,5}$=2.01 Hz, 3-H), 12.59 (1H, s, 4'-OH).

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