

Chemical and Pesticidal Studies on *Acorus Calamus* Rhizomes

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Abstract: 2,4,5-trimethoxy benzaldehyde was isolated from the petroleum ether extract of *Acorus calamus* rhizomes. The structure of the compound was confirmed by chemical and spectroscopic techniques including high resolution ¹H-NMR, ¹³C-NMR, H-H Cosy and HMBC. Based on toxicity data on percentage mortality and corrected mortality due to the effect of *Acorus calamus* rhizome extract with petroleum ether, acetone and methanol against the adults of *Tribolium confusum* (flour beetle) and *Sitophilus oryzae* (Rice weevil), it was observed that petroleum ether extract of the rhizome was more toxic than that of other extracts in the insect species. From the repellency class it was observed that the petroleum ether extract was more effective against *Tribolium castaneum* than *Tribolium confusum*.

Key words: 2,4,5-trimethoxybenzaldehyde, pesticidal, *acorus calamus*, rhizomes

INTRODUCTION

Acorus calamus is one of the most important medicinal plants of Araceae family. Locally it is known as “Gora bach or White bach” and is distributed all over Bangladesh^[1]. This plant is reported to be useful for insecticide^[2]. The results of the previous phytochemical investigation of this taxon have been reported^[3,4,5]. Although it has previously been investigated by different research groups but the findings are found to differ from place to place of the origin of plants. This variation may be due to difference of soil constituents, climate and time of collection. Different parts of the plant are used for the treatment of various diseases. In Ayurvedic medicine, the rhizomes are considered to possess anti-spasmodic, carminative and anthelmintic properties and are used for the treatment of epilepsy, chronic diarrhea, dysentery, bronchial catarrh and abdominal tumours^[6]. They are also employed for kidney and liver troubles, rheumatism and eczema^[6].

To alleviate insect pest problems in storage, synthetic pesticides are generally recommended. These may have drawbacks, such as the development of genetic resistance, toxicity to non-target organisms, residual toxicity, increasing cost of application, environmental pollution, hazards from handling^[7] etc. This has created a worldwide interest in the development of alternative strategies, including the use of new types of insecticides derived from a re-evaluation of age-old, traditional botanical pest control agent (8). The main advantages of botanicals are that

they are less expensive and comparatively safer to mammals and higher animals^[9]. Of late effectiveness of derivatives of several botanical families as deterrent and growth inhibitor against stored grain insects has also been reported^[10,11]. The literature survey showed that the extract of *Acorus calamus* had significant insecticidal activity^[12,13,14] and it was almost left uninvestigated in Bangladesh in spite of possessing insecticidal potency. This makes us more interested to isolate the main constituents of this plant having insecticidal activity. A systematic study on the plant was undertaken and the work was extended to investigate on petroleum ether extract.

The mature plants were collected from Chittagong, the southern part of Bangladesh. The analysis was mainly worked on the rhizomes of the plant. Here, we report, the isolation of 2,4,5-trimethoxy benzaldehyde from *Acorus calamus* and pesticidal activities of the plant extracts.

MATERIALS AND METHODS

General Experimental Procedures: IR spectra were recorded as dry film on a Mattson Galaxy 5000 FT-IR spectrometer. HREIMS were obtained on a JEOL JMS-AX505HA double-focusing instrument at 70 eV. NMR spectra (both 1D and 2D) were acquired on a Bruker AMX-400 (400 MHz for ¹H and 100 MHz for ¹³C) spectrometer using the residual solvent peaks as internal standard. J-modulated ¹³C spectra were acquired with a relaxation time (d1) of 6s. HMBC spectra were optimized for a long range JH-C of 7Hz

($d_6=0.07s$). Vacuum Liquid Chromatography (VLC) was carried out on short column packed with TLC grade silicagel (Kieselgel 60H, Merck) that was operated under reduced pressure. Column Chromatography (CC) was carried out using Merck Si gel (mesh 70-230). Preparative Thin Layer Chromatography (PTLC) was conducted by using Merck Silicagel 60 PF254 on glass plates (20cmX20cm) at a thickness of 0.5 mm. Thin Layer Chromatography (TLC) was carried out on normal-phase Merck Silicagel 60 PF254 plates. Spots on TLC and PTLC plates were visualized after spraying with 1% vanillin- H_2SO_4 followed by heating at 110°C for 5-10 min.

Plant Material: The mature plants were collected from Chittagong in March, 2006. The analysis was mainly worked on the rhizomes of the plant. The rhizomes were cut into small pieces and dried in shed.

Extraction and Isolation: The dried rhizomes were crushed (200 mesh) by using a cyclotech grinding machine. After wards the dried rhizome powder (1 kg) was taken in a few pre-cleaned cloth bags and extracted sequentially with petroleum ether (40°-60°C), acetone and methanol using a Soxhlet's apparatus. The extraction process was carried out by refluxing the solvent for several hours and about twenty hours were required for the process. The extracts were collected and filtered individually and concentrated under reduced pressure using a rota-vapor. The concentrated petroleum ether extract was mixed with a small amount of column grade silica gel (70-230 mesh, E-MERCK) maintaining the ratio, concentrated mass: silica gel = 2:1 and dried in air. After drying, the mixture was powdered in a mortar. This powder was then ready for fractionation by column.

The petroleum ether extract was subjected to CC using mobile phase toluene and chloroform. The elutes were combined together on the basis of TLC analysis. CC fraction eluted with 10% Chloroform in toluene was subjected to PTLC (solvent system, 10% Chloroform in toluene) to obtain compound ACC. The plates were developed in a solvent tank containing solvent system, toluene: chloroform (9:1). The separated bands were visualized by the use of UV light (350 nm). The lower UV active band was separated to get the compound ACC (46mg). This compound was dissolved in $CHCl_3$. The TLC plate over silica gel G-60, PF₂₅₄ was developed in a solvent tank containing solvent system, toluene: chloroform: ethyl acetate (5:3:2). The plate was taken out from the solvent tank and dried in the air. Under UV light a beautiful glow at 350 nm (R_f 0.49) was observed.

After extraction, the concentrated extract was preserved in a small reagent bottle at 40°C in refrigerator to perform the pesticidal activity. Stock culture of *Sitophilus oryzae* and *Tribolium confusum* were maintained in earthen pot (18x9.5cm) on sterilized wheat in 30°C±0.5°C in an incubator. For testing beetle mortality, six doses were used including control (only solvent). Fifteen days old beetles of pure culture of *Sitophilus oryzae* and *Tribolium confusum* were collected from the laboratory of Applied Zoology Division BCSIR, Rajshahi. The experimental doses 0.0025% (0.0094 mg/cm²), 0.125% (0.0196 mg/cm²), 0.25% (0.0393 mg/cm²), 0.5% (0.078 mg/cm²) and 1% (0.157 mg/cm²) were taken. The doses were prepared by mixing the requisite quantities of extracted materials with 10 ml acetone or methanol. Methanol was used in the case of methanolic extract because this extract was not dissolved properly in acetone. Five doses were selected for each extract depending on the toxic nature of the plant. The experiments were carried out by adopting the method of residual film technique. For each test dose, 1 ml liquid was dropped on a Petri-dish (9.5 cm diameter). After drying four plastic rings (30 mm) were placed inside the Petri-dish and 10 adults were released in each ring. Each ring represented a replication. The doses were calculated by measuring the weight of extracted materials (mg) in 1 ml of solvent divided by the surface area of the Petri-dish and it is converted into mg/cm². Mortality was assessed after 24 hours, 48 hours and 72 hours of treatment. The mortality was corrected by using Abbott's formula (15) and L_{D50} values were determined by Probit analysis (16). The experiments were replicated thrice and were performed at 30°C ± 1°C.

RESULTS AND DISCUSSION

The compound ACC was isolated as a pale yellowish amorphous solid material from the petroleum ether extract of the rhizome powder of *Acorus calamus*. It melted at 95-98°C. The TLC examination of ACC indicated that it was a single compound (R_f 0.49, toluene:chloroform:ethyl acetate=5:3:2 as a developing solvent). Its TLC behaviour showed a yellowish coloured spot under UV light. No response was obtained in iodine vapour.

The IR of the compound ACC had absorption frequency at 1734 cm⁻¹ due to carbonyl group (>C=O) stretching. The peak at 1656 cm⁻¹ was due to aromatic >C=C< stretching which indicated that the presence of aromatic ring in the compound. The absorption band at 2920 cm⁻¹ was due to C=C-H stretching and peak at 824 cm⁻¹ was due to C-H which might be present in benzene ring. The presence of C-O stretching was suspected from the absorption peak at 1128 cm⁻¹.

Table 1: ¹H-NMR data of ACC.

Position of hydrogens	Chemical shift (ppm)
H-3	6.5 (S, 1H)
H-6	7.32 (S, 1H)
H-7	10.32 (S, 1H)
H-8	3.97 (S, 3H)
H-9	3.92 (S, 3H)
H-10	3.87 (S, 3H)

Table 2: ¹³C-NMR data of ACC.

Carbon number	δc vlues in ppm
C-1	117.49
C-2	143.73
C-3	96.09
C-4	158.83
C-5	155.96
C-6	109.15
C-7	188.22
C-8	56.47
C-9	56.40
C-10	56.39

Table 3: Important H-H COSY interaction in ACC.

H-8 (3.97)	↔	H-2 (6.5)	↔	H-9 (3.92)
H-6 (7.32)	↔	H-10 (3.87)		

Table 4: Important HMBC interaction in ACC.

Protons	Carbons
H-7 (10.32)	→ C-1 and C-6
H-3 (6.5)	→ C-2, C-4 and C-5
H-6 (7.32)	→ C-1, C-5 and C-7

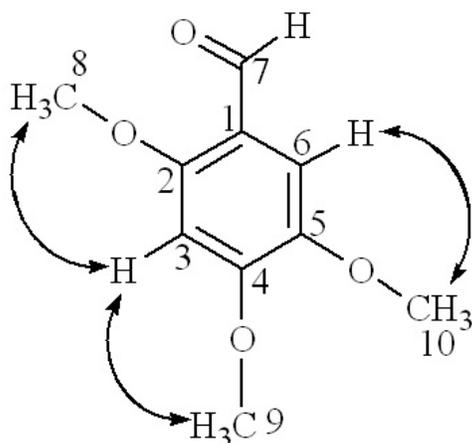


Fig. 1: H-H Cosy interaction in ACC

In the ¹H-NMR of the compound ACC showed that it might be 2,4,5-trimethoxy benzaldehyde. A sharp singlet at 10.32 ppm was assigned to the aldehyde proton. Two peaks at 6.5 and 7.32 ppm were due to phenyl protons (H-3 and H-6 respectively) situated at 1,4 position to each other. Three sharp peaks at 3.97, 3.92 and 3.87 ppm were due to C=O group at C-2, C-4 and C-5 respectively. The ¹H-NMR values are recorded in table 1.

The ¹³C-NMR revealed the presence of about 10 carbons in ACC. The signal at 188.22 ppm was due to C=O group. The other ¹³C-NMR signals were

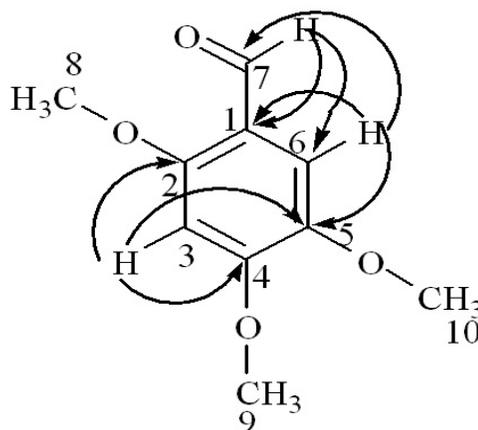
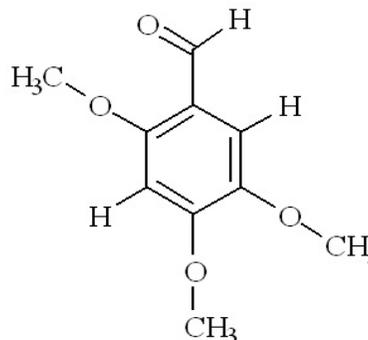


Fig. 2: HMBC interaction in ACC



2,4,5-trimethoxy benzaldehyde

Fig. 3: Structure of the compound 2,4,5-trimethoxy benzaldehyde

comparable to the reported data of 2, 4, 5- trimethoxy benzaldehyde. Against each of the carbon numbers the ¹³C-NMR values are recorded in table 2.

Important H-H Cosy interactions in ACC are recorded in table-3. The arrangement of protons in the compound ACC was confirmed from the H-H Cosy experiment and recorded in figure 1.

In H-H Cosy experiment, there was a strong cross peak between protons resonating at 7.32 (H-6) and 3.87 (H-10) ppm, so they were adjacent to each other. A strong cross peak between two protons resonating at 3.97 (H-8) and 6.5 (H-3) ppm indicate that they were adjacent to each other. Another strong cross peak between two protons resonating at 6.5 (H-3) and 3.92 (H-9) ppm indicate that they were also adjacent to each other. Therefore two methoxy groups (-O-CH₃) were situated at 1,3-position to each other.

The arrangement of the carbons in the structure of ACC was further confirmed by the long-range C-H correlation spectroscopy (HMBC). The important HMBC interactions in ACC are recorded in table 4 and figure 2.

Table 5: Effect of different plant materials on adult mortality of *Tribolium confusum*

Plant material	Duration after treatment	Dose mg/cm ²	Petroleum ether extract		Acetone extract		Methanol extract	
			Percentage mortality	Corrected mortality	Percentage mortality	Corrected mortality	Percentage mortality	Corrected mortality
<i>Acrus calamus</i> (White bach Richizome)	24 hours	0.0094	57.5	58	15	15	N. E.	N. E.
		0.0196	70	70	49.17	49	N. E.	N. E.
		0.0393	82.5	83	60	60	N. E.	N. E.
		0.078	90.83	91	75	75	N. E.	N. E.
		0.157	95	95	90	90	N. E.	N. E.
	48 hours	0.0094	60.83	61	20	20	N. E.	N. E.
		0.0196	87.5	88	55	55	N. E.	N. E.
		0.0393	90	90	67.5	68	N. E.	N. E.
		0.078	95	95	87.5	88	N. E.	N. E.
		0.157	98.33	98	98.33	98	N. E.	N. E.
	72 hours	0.0094	78.33	78	50	50	N. E.	N. E.
		0.0196	95	95	65	65	N. E.	N. E.
		0.0393	98.33	98	80	80	N. E.	N. E.
		0.078	99.17	99	92.5	93	N. E.	N. E.
		0.157	99.17	99	99.17	99	N. E.	N. E.

N.E. = No effect

Table 6: Effect of different plant materials on adult mortality of *Sitophilus oryzae*

Plant material	Duration after treatment	Dose mg/cm ²	Petroleum ether extract		Acetone extract		Methanol Extract	
			Percentage mortality	Corrected mortality	Percentage mortality	Corrected mortality	Percentage mortality	Corrected mortality
<i>Asprus Calamus</i> (White bach rhizome)	24 hours	0.0094	16.67	17				
		0.0196	48.33	48				
		0.0393	85	85	12.5	13	7.5	8
		0.078	99.17	99	37.5	38	12.5	13
		0.157	99.17	99	60	60	12.5	13
		0.314			97.5	98	15	15
		0.471			99.17	99	27.5	28
	48 hours	0.0094	50	50				
		0.0196	70	70				
		0.0393	95	95	22.5	23	62.5	63
		0.078	99.17	99	42.5	43	72.5	73
		0.157	99.17	99	71.67	72	82.5	83
		0.314			98.33	98	92.5	93
		0.471			99.17	99	99.17	99

Table 6: Continued

72 hours	0.0094	85	85				
	0.0196	96.67	97				
	0.0393	99.17	99	50	50	72.5	73
	0.078	99.17	99	65	65	85	85
	0.157	99.17	99	80	80	91.67	92
	0.314			92.5	93	97.5	98
	0.471			99.17	99	99.17	99

Table 7: Toxicity of different solvent extracts on *Tribolium confusum*.

Plant material	Duration after treatment	Solvent used	χ^2 for heterogeneity	Regression equation	LD ₅₀ mg/cm ²	Fiducial limits	
						Lower	Upper
<i>Achras calamus</i> (White bach rihzome)	24 hours	Pet. ether	0.479	Y=3.238+3.813X	0.0068	0.004	0.010
		Acetone	7.374	Y=2.558+1.688X	0.027	0.023	0.032
		Methanol	N.E	N.E	N.E	N.E	N.E
	48 hours	Pet. ether	4.596	Y=4.014+1.414X	0.0049	0.003	0.007
		Acetone	5.365	Y=2.146+2.149X	0.021	0.018	0.024
		Methanol	N.E	N.E	N.E	N.E	N.E
	72 hours	Pet. ether	5.795	Y=4.217+1.667X	0.003	0.003	0.005
		Acetone	1.914	Y=3.264+1.690X	0.010	0.008	0.013
		Methanol	N.E	N.E	N.E	N.E	N.E

Table 8: Average repellence of *Acorus calamus* rhizome extract (Pet.ether) to red flour beetle (*T. castaneum*) and confused flour beetle (*T. confusum*) using treated filter paper test.

Insect	Concentration of extract µg/cm ²	Average repellence (%) at hours after treatment					Mean	Repellence class
		1	2	3	4	5		
<i>T. castaneum</i>	78.60	100	100	100	86.66	80.00	93.00	V
	314.38	100	93.34	100	93.34	93.34	96.00	V
	628.76	100	100	100	93.34	100	98.67	V
<i>T. confusum</i>	78.60	40	73.34	66.66	66.66	86.66	66.67	IV
	314.38	66.67	60.00	66.66	60	80	66.67	IV
	628.76	40	80	93.34	66.66	86.66	69.33	IV

The proton H-7 had cross peak due to two-bond coupling with C-1 and three-bonded coupling with C-6. The aromatic proton H-3 had cross peak due to two bonded coupling with C-2 and C-4 and three bonded coupling with C-5. The aromatic proton H-6 had also cross peak due to two bonded coupling with C-1 and C-5 and three bonded coupling with C-7. Thus C-H correlation spectrum (HMBC) further supported the structure of ACC.

From the analyses of the ¹H-NMR, ¹³C-NMR, H-H Cosy and HMBC spectra, the compound ACC was

identified as 2, 4, 5-trimethoxy benzaldehyde. Although it is a known organic compound this is the first report of its occurrence in the genus *Acorus*.

Basic toxicity data on percentage mortality and corrected mortality due to the effect of *Acorus calamus* rhizome extracted with petroleum ether, acetone and methanol on the adults of *Tribolium confusum* (flour beetle) and *Sitophilus oryzae* (Rice weevil) are summarized in Table 5 & 6. From this result it is observed that petroleum ether extract of the rhizome was the most toxic than that of other extraction in both

the insect species. Their efficacy followed the order petroleum ether> acetone> methanol.

Results due to the effect of petroleum ether, acetone and methanol extracts of *Acorus calamus* rhizomes against *Tribolium confusum* are shown in table 7. The pet ether extract of *Acorus calamus* rhizome exhibited lowest L_{D50} values i.e, the relative toxicity was highest in respect of the mortality records for 24, 48 and 72 hours after treatment and their efficacy followed the order: ether> acetone> methanol.

The repellent effect of the most effective toxicants against one to two week old adult beetle of *Tribolium castaneum* (Red flour beetle) and *Tribolium confusum* (Confused flour beetle) are summarized in Table 8. All the doses of *A. calamus* rhizome extract (pet. ether) showed effective repellency to both the insect species. From the repellency class it is observed that the extract was more effective to *T. castaneum* than *T. confusum*.

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