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Chemical And Pesticidal Studies On *Acorus Calamus* Rhizomes

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ABSTRACT

2,4,5-trimethoxy banzaldehyde was isolated from the petroleum ether extract of *Acorus calamus*. The structure of the compound was confirmed by chemical and spectroscopic techniques including high resolution ^{1}H-NMR, ^{13}C-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 pet ether extract was more effective to *Tribolium castaneum* than *Tribolium confusum*.

KEYWORDS

2,4,5-trimethoxy banzaldehyde;
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 diffrent 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 G.H.Schmidt and M.Streloke 1994. Effect of *Acorus calamus*(L.)(Araceae)oil and its main compound β-Asarone on *Prostephamus truncatus*(Horn)(coleoptera: Bostrichidae). J.Stored Prod. Res., 30: 227-235.antispasmodic, carminative and anthemintic properties and are used for the treatment of epilepsy, chronic diarrhoea, dysentry, 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 handing[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 mail 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 try the isolation, identification and examination of insecticidal activity of the main constituents of this plant. 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 analysis was mainly worked on the rhizomes of the plant. Here, we report, the isolation of 2,4,5-trimethoxy banzaldehyde from *Acorus calamus* and pesticidal activities of 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 6 s. HMBC spectra were optimised for a long range JH-C of 7Hz(d6=0.07s). Vacuum Liquid Chromatography (VLC) was carried out on short column packed with TLC grade silica gel (Kiselgel 60H, Merck) that was operated under reduced pressure. Column chromatography(CC) was carried out using Merck Si gel(mesh 70-230). PTLC was conducted by using Merck Si gel 60 PF254 on glass plates(20cm×20cm) at a thickness of 0.5mm. TLC was carried out on normal-phase Merck Si gel 60 PF254 plates. Spots on TLC and PTLC plates were visualized after spraying with 1% vanillin-H2SO4 followed by heating at 110^0C for 5-10min.

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 sunlight.

Extraction and isolation

The dried rhizomes were ground(200 mesh) by using a cyclotech grinding machine. Afterwads the dried rhizome powder(1kg) was taken in a few precleaned cloth bags and extracted sequentially with petroleum ether(40^0-60^0C), acetone and methanol using a Soxhlet 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. All extracts were 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, concentration mass: silica gel=2:1 and dried in air. After drying the mixture was powdered in a morter. This powder was then ready for fractionation by column.

The petroleum ether extract was subjected to CC using mobile phase toluene and chloroform. The eluates 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(350nm). The lower UV active band was separated a compound ACC(46mg). This compound was dissolved in $CHCl_3$. The TLC plate over silica gel G-60, PF_{254} was developd in a solvent tank containing solvent system, toluene: chloroform: ethyl

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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 350nm(R_f 0.49) was observed.

After extraction, concentrated extract collected in a small reagent bottle, preserved at 40^{0}C in refrigerator to perform the pesticidal activity. Stock culture of *Sitophilus oryzae* and *Tribolium confusum* were mainted in earthen pot (18×9.5cm) on sterilized wheat in 30^{0}C±0.5^{0}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%(0.0094mg/cm^2), 0.125%(0.0196mg/cm^2), 0.25%(0.0393mg/ cm^2), 0.5%(0.078mg/cm^2) and 1%(0.157mg/cm^2) 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 1ml liquid was dropped on a petridish(9.5cm diameter). After drying four plastic rings(30mm) were placed inside the petridish 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 1ml of solvent divided by the surface area of the petridish and it is converted into mg/cm^2. Mortality was assessed after 24 hours, 48 hours and 72 hours of treatment. The mortality was corrected by using Abbott's formula[15] and LD50 values were determined by Probit analysis[16]. The experiments were replicated thrice and were performed at 30^{0}C±1^{0}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^{0}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 1734cm^{-1} due to carbonyl group(>C=O) stretching. The peak at 1656cm^{-1} was due to aromatic >C=C< stretching which indicated that the presence of aromatic ring in the compound. The absorption band at 2920cm^{-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 1128cm^{-1}.

In the ^{1}H-NMR of the compound ACC showed that it might be 2,4,5-trimethoxy benzaldehyde. A sharp singlet at 10.32ppm was asigned 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 3.87ppm were due to-O-C$\underline{H}_3$ group at C-2, C-4 and C-5 respectively. The ^{1}H-NMR values are recorded in TABLE 1.

The ^{13}C-NMR revealed the presence of about 10 carbons in ACC. The signal at 188.22 ppm was due to C=O group. The other ^{13}C-NMR signals were comparable to the reported data of 2, 4, 5-trimethoxy benzaldehyde. Against each of the carbon numbers

TABLE1: ^{1}H-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 : ^{13}C-NMR data

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

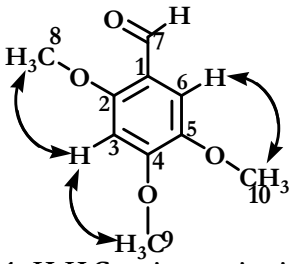

Figure 1 : H-H Cosy interaction in ACC

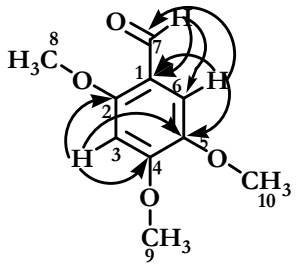

Figure 2 : HMBC interaction in ACC

the ^{13}C-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-CH_3) 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.

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 ^{1}H-NMR, ^{13}C-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*.

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

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

Plant material	Duration after treatment	Dose mg/cm²	Petroleum ether extract		Acetone extract		Methanol extract	
			Percentage mortality	Corrected mortality	Percentage mortality	Corrected mortality	Percentage mortality	Corrected mortality
Acorus calamus (White bach rhizome)	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

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

Plant material	Duration after treatment	Dose mg/cm²	Petroleum ether extract		Acetone extract		Methanol Extract	
			Percentage mortality	Corrected mortality	Percentage mortality	Corrected mortality	Percentage mortality	Corrected mortality
Acorus Calamus (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
	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/cm²	Fiducial limits	
						Lower	Upper
Acorus calamus (White bach rhizome)	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

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.

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/cm²	Average repellence (%) at hours after treatment					Mean	Repellence class
		1	2	3	4	5		
T. castaneum	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
T. confusum	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

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 LD50 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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