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ISOLATION OF PHYTOCONSTITUENTS FROM THE ROOTS OF BAMBUSA ARUNDINACEA

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ABSTRACT

The phytochemical examination of the roots of *Bambusa arundinacea*. The air dried powdered plants materials were separately extracted successively with petroleum ether (60^0–80^0C) and ethanol using a soxhlet extractor. The solvents were purified by distillation prior to extraction. The period of extraction was fixed at 48 h for every solvent at every stage of the extraction process. The ethanol extract of *Bambusa arundinacea* roots upon concentration under reduced pressure left a greasy with brown colour residue (7.2%) which gave positive ferric and Shinoda tests for flavonoids and negative Liebermann-Burchard reaction. TLC examination of the residue over silica gel- G showed number of spots in solvent system: ethyl acetate: ethanol-1: 0.25 on spraying with 5% alcoholic H_2SO_4 followed by heating. The ethanol residue was subjected to column chromatography over silica gel (120 mesh). Fractions of 200 ml were collected. The residue was subjected to column chromatography over silica gel afforded three compounds named HKS-04, HKS-05 and HKS-06. The compounds were characterized through chemical and spectral analysis and confirmed as 3', 3, 6, 7-tetramethoxy-4', 5, 8-trihydroxy flavone (HKS-04), 4-methoxy benzoic acid (HKS-05) and 4'-hydroxy flavan-3-ol (HKS-06) respectively.

KEYWORDS: *Bambusa arundinacea*, 3', 3, 6, 7-tetramethoxy-4', 5, 8-trihydroxy flavone (HKS-04), 4-methoxy benzoic acid (HKS-05) and 4'-hydroxy flavan-3-ol (HKS-06).

INTRODUCTION

Bambusa arundinacea of family poaceae is distributed throughout India, except Himalaya and Indo-Gangetic plain. The plant is large densely caespitose thorny bamboo with curving branches form a thick rootstocks; culms bright green, shining, up to 24 to 30m high and 15-18 cm diameter, branches from the base, the lower joints giving out long horizontal shoots armed at the 73 nodes with 2-3 recurved thorns and with few leaves. Leaves linear-laceolate or linear, 12-20 x 1.2-1.8 (2.5) cm, rounded at the base into a short, 2.5 mm petiole, glabrous above except for long hairs near the base, glabrous or puberulous beneath, scabrous on one or both margins and ciliate towards the base; leaf-sheath striate, glabrous or slightly pubescent, ending in a thick, often ciliate callus and a short auricle furnished with a few stiff, curved, white, deciduous bristles, edges ciliate; ligule short. Panicle often occupying the whole plant.[1-3]

Young shoots of the bamboo made into a poultice is a most efficacious application for dislodgement of worms from ulcers. Leaf bud is administered in decoction to encourage the free discharge of the menses or lochia after delivery when it is scanty.[4] Used in leprosy, fevers and haemoptyis, and also in case of children suffering from thread worms. Pickles or curry preparedout of the tender shoots give much benefit to persons suffering from lack of digestion as it promotes appetite and digestion. The silicious concretion as found in the joints of the female bamboo, it is useful in fever, cough, consumption, paralytic complaints, debilitating diseases, asthma, snake-bite, etc. Root is given as a specific in eruptive affections.[5,6]

In the present work, we have isolated 3', 3, 6, 7-tetramethoxy-4', 5, 8-trihydroxy flavone (HKS-04), 4-methoxy benzoic acid (HKS-05) and 4'-hydroxy flavan-3-ol (HKS-06) from the ethanol extract of dried roots of *Bambusa arundinacea*.

MATERIALS AND METHODS

Plant Material

The fresh plant materials of *Bambusa arundinacea* roots was collected from young matured plants and authenticated. After authentication, the plant materials were collected in bulk, washed under running tap water to remove adhering dirt followed by rinsing with distilled water. The plant materials were then shade dried and

separately pulverized in a mechanical grinder followed by sieving (sieve no. 40) to obtain coarse powder.

Preparation of Extract and Isolation

The dried powdered roots (500 g) were separately extracted successively with petroleum ether (60^0–80^0C) and ethanol using a soxhlet extractor. The period of extraction was fixed at 48 h for every solvent at every stage of the extraction process. The solvents were purified by distillation prior to extraction. After completion of extraction, the liquid extracts were concentrated under vacuum to yield dry extracts. The above extracts were used for further studies such as colour, consistency and extractive values. Fluorescence characteristics of liquid extracts were observed under daylight and ultraviolet light separately at short and long wavelengths.[7] Standard methods[8-10] were used for preliminary phytochemical screening of the different extracts to know the nature of phytoconstituents present within them. The results are depicted in the tables (Table 1 to 3).

The ethanol extract of *Bambusa arundinacea* roots upon concentration under reduced pressure left a greasy with brown colour residue (7.2%) which gave positive ferric and Shinoda tests for flavonoids and negative Liebermann-Burchard reaction. TLC examination of the residue over silica gel- G showed number of spots in solvent system: ethyl acetate: ethanol-1: 0.25 on spraying with 5% alcoholic H_2SO_4 followed by heating. The ethanol residue was subjected to column chromatography over silica gel (120 mesh) using n-Hexane: benzene; benzene: chloroform; chloroform: ethanol at different ratio. Fractions of 200 ml were collected each time.

RESULT AND DISCUSSION

The structures of the compound isolated were elucidated on the basis of spectroscopic methods and are given in Fig no.1.

Compound HKS-04

The fractions 73 to 80 [Benzene: Chloroform (30:70)] were found to be similar on TLC and hence combined and recrystallized from petroleum ether when colourless needles obtained, melting point 178-180^0 C (100 mg). Rf value 0.47 (n-Hexane: Diethyl ether-1:1). Shinoda test: pink colour.

The IR (v cm^{-1}) spectrum of compound 4 (HKS-04) showed absorption bands at 3608.9 to 3315.7 (O-H, free hydroxyl group), 2953.1 (Cyclic C-H, str), 2866.3 (Ali-C-H, str), 1660.7 (C=O), 1500.0-1400.3 (C-C ring stretch), 1284.6-1193.8 (C-C stretching), 1114.8-997.2 (O-H, out of plane bend).

The ^{1}H-NMR spectrum of compound displayed the characteristic signals at δ_H 7.80 (H-2', s), 7.30 (H-5', d), 6.67 (OH-4', s), 5.89 (OH-5, s), 4.29 (OH-8, s), 4.17 (OCH_3-3, s) 3.85 (OCH_3-3', s), 3.14 (OCH_3-6, s), 2.37 (OCH_3-7).

The ^{13}C-NMR spectrum of compound displayed the characteristic signals at δ_H 2-77.37, 3-126.99, 4-123, 5-135.55, 6-140.11, 7-145.84, 8-105.20, 3'-148.77, 1'-100.62, 2'-77.00, 5'-76.57, 6'-64.21.

The mass data which showed m/z = 390 indicative of **$C_{19}H_{18}O_9$,** m/z = 358 **$C_{18}H_{14}O_8$,** m/z = 334 **$C_{16}H_{14}O_8$,** m/z = 304 **$C_{15}H_{12}O_7$,** m/z = 212 **$C_9H_8O_6$,** m/z = 98 **$C_5H_6O_2$,** m/z = 79 **C_5H_3O,** m/z = 42 **C_2H_2O.**

Compound 4 was isolated and its molecular formula was determined as $C_{19}H_{18}O_9$ (m/z = 390 (100) [M^+]). The structures of the flavone were identified on the basis of extensive spectroscopic data analysis and by comparison of their spectral data with those reported in the literature. The IR spectrum indicated the presence of hydroxyl (3608.9 cm^{-1}) and carbonyl functions (1660.7 cm^{-1}). The occurrence of a flavone skeleton in the molecule could be easily deduced from the ^{1}H-NMR and ^{13}C-NMR spectrums. From the above mentioned data of ^{13}C NMR signal indicated the presence of hydroxyl group at C-5, C-8 and C-4'and unsaturated keto function. The ^{13}C NMR signal also reported the presence of four methoxy group at different position of flavones skeleton. The ^{13}C-NMR signal of different location of carbon for functional group was confirmed by the signal of were confirmed spectra of ^{1}H-NMR. The ^{1}H-NMR further showed the presence of three hydroxyl, four methoxyl group and three methine group. The compound characterized as 3', 3, 6, 7-tetramethoxy -4', 5, 8-trihydroxy flavones.

Compound HKS-05

Fractions 90 to 96 were mixed as they were similar and on crystallization from aqueous ethanol, a yellow crystalline solid was obtained melting point 206-208^oC (110 mg). Rf value 0.64 in ethyl acetate: ethanol-1: 1.25.

The IR (v cm^{-1}) spectrum of compound 5 (HKS-05) showed absorption bands at 3063.0 to 3030.2 (Aromatic C-H, str), 2974.3 (Cyclic C-H, str), 2881.7 to 2816.1 (Ali- C-H, str), 1683.9 (COOH str), 1629.9 (C=C stretch), 1579.7 (Asymmetric carboxylate anion), 1494.8 to 1448.5 (C-C ring stretch), 1419.6 (C-C ring stretch), 1099.4 to 1072.4 ((-CO, stretch), 979.8, 918.1, 958.6 to 871.8 (O-H, out of plane bend), 644.2 to 532.7 (Out of plane C=C).

The ^{1}H-NMR spectrum of compound displayed the characteristic signals at δ_H 7.73 (COOH-1, s), 7.435 (H-2, d), 6.582 & 6.52 (H-3&5,d), 2.06 (OCH_3-4,s), 7.45 (H-6,d).

The ^{13}C-NMR spectrum of compound displayed the characteristic signals at δ_H 1-206.58, 2-119.00, 3-128.95, 4-145.64, 5-131.07, 6-129.71.

The mass data which showed m/z = 200 indicative of **$C_{12}H_8O_3$,** m/z = 136 **$C_8H_8O_2$,** m/z = 126 **$C_6H_6O_3$,** m/z = 120 **$C_7H_4O_2$,** m/z = 103 **C_7H_3O,** m/z = 96 **$C_5H_4O_2$,** m/z = 95 **$C_5H_3O_2$,** m/z = 77 **C_5HO,** m/z = 50 **C_4H_2.**

Compound 5 was isolated and its molecular formula was determined as $C_{12}H_8O_3$, (m/z 152) by mass spectrometry. The IR spectrum contained an absorption band due to COOH at 1683.9 cm^{-1}. The ^{13}C NMR spectrum showed 12 carbon signals, including four methines. The four methine at 2,3,5 & 6. One carbon of the benzene ring was substituted by a carboxylic acid group and methoxy group. The ^{1}H NMR spectrum exhibited 8 proton signals. The structures of these known 4-methoxy benzoic acid were identified on the basis of extensive spectroscopic data analysis and by comparison of their spectral data with those reported in the literature.

Compound *HKS-06*

The fractions 97 to 101 showed similar spots in TLC hence combined and crystallized from alcohol which resulted in a yellow crystalline solid, m.p. 168-170^0 C (82 mg). It appeared deep purple under UV light and changed to yellow when exposed to ammonia. Shinoda's test: Magenta colour. Rf value 0.74 in Chloroform: Ethyl acetate-2:1.

The IR (v cm^{-1}) spectrum of compound 6 (HKS-06) showed absorption bands at 3525.9 to 3427.6 (O-H, free hydroxyl group), 3321.5 (O-H, str), 2943.4 (Cyclic C-H, str), 1699.3 (C=C stretch), 1452.4 (C-C ring stretch), 1244.1 (C-C stretching), 1074.3 (-CO, stretch), 943.2, 895.0, 868.0 (O-H, out of plane bend), 661.6, 636.5 to 570.9 (Out of plane C=C).

The ^{1}H-NMR spectrum of compound displayed the characteristic signals at δ_H 7.915 (OH-4', s), 4.624 (H-3, q), 4.595 (H-2, d), 4.282 (H-6, t), 3.945 (H-7, t), 3.833 (H-8, d), 3.746 (H-4, d), 3.556 (H-5, d), 3.199 (H-5', d), 2.008 (H-6', d), 3.160 (H-3',d).

The ^{13}C-NMR spectrum of compound displayed the characteristic signals at δ_H 2-78.23, 3-129.23, 4-73.95, 5-74.73, 6-75.70, 7-79.40, 8-80.33, 3'-80.92, 4'-144.02, 1'-180.32, 2'-77.22, 6'-123.95, 5'-105.75.

The mass data which showed m/z = 242 indicative of **$C_{15}H_{14}O_3$,** m/z = 224 **$C_{15}H_{12}O_2$,** m/z = 216 **$C_{13}H_{12}O_3$,** m/z = 200 **$C_{13}H_{12}O_2$,** m/z = 132 **C_9H_8O,** m/z = 105 **C_7H_5O,** m/z = 81 **C_5H_5O,** m/z = 68 **C_4H_4O,** m/z = 43 **C_3H_3O.**

Compound 6 was obtained and gave EI MS spectrum of molecular ion peak at m/z 242, corresponding to the molecular formula $C_{15}H_{14}O_3$, supported also by spectroscopic analysis. In the ^{1}H NMR spectrum, the double doublets were observed for methine proton at 5, 6, 7, 8, 2', 3', 5' & 6' position of ring. The singlet for proton of hydroxyl group was found at 3 & 4' of carbon, and these signals are typical of a flavonoid nucleus with an unsubstituted ring. The double doublets protons at 2', 3', 5' & 6' of carbon suggested that third ring was saturated. This splitting pattern was due to the coupling between the H-2 axial proton and the H-3 geminal protons. The above data suggested a flavanone nature for compound 9. From the above data of ^{13}C NMR of δ supported the presence of two hydroxyl group and while other were CH and CH_2 groups. The data of IR supported the presence of hydroxyl group and saturated ring. Structures were elucidated on comparison with data in the literature was 4'-hydroxy flavan-3-ol.

Table 1: Data showing the colour, consistency and extractive values of extracts of the *Bambusa arundinacea root.*

Plant	Parts used	Extract	Colour	Consistency	Yield % w/w
Bambusa arundinacea	Roots	Petroleum ether	Pale green	Waxy and oily	2.1
		Ethanol	Brown	Greasy	7.2

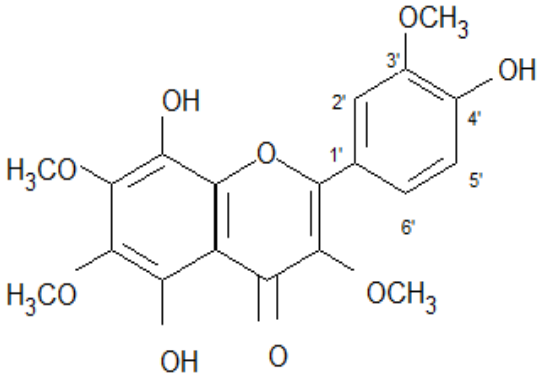
Table 2: Fluorescence characteristics of extracts of the *Bambusa arundinacea* under daylight and UV light.

Plant	Parts used	Extract	Colour		
			Day light	Short uv	Long uv
Bambusa arundinacea	Roots	Petroleum ether	Pale green	Pale green	Pink
		Ethanol	Brown	Green	Red

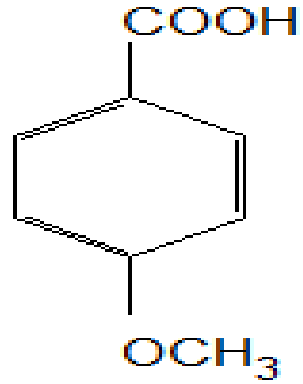
Table 3: Preliminary phytochemical screening of petroleum ether and ethanol extracts of *Bambusa arundinacea* roots.

Extract	Alkaloids	Carbohydrates	Gums and mucilages	Proteins and amino acids	Tannins and phenolic compounds	Steroids and sterols	Triterpenoids	Saponins	Flavonoids
Pet. Ether	-	-	+	-	-	+	+	-	-
Ethanol	-	+	-	+	+	-	-	+	+

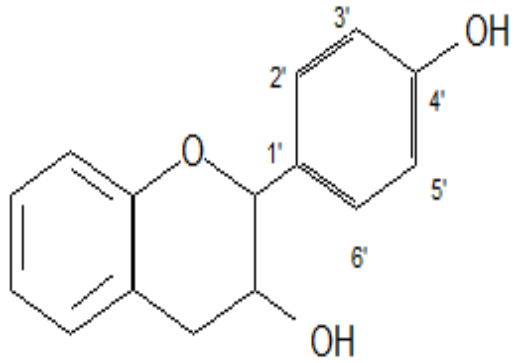
(+): Present; (-): Absent.



(HKA-04) 3', 3, 6, 7-tetramethoxy -4', 5, 8-trihydroxy flavone.



(HKS-05) 4-methoxy benzoic acid.



(HKS-06) 4'-hydroxy flavan-3-ol.

Fig.1: Structure of the compounds isolated from the ethanol extract of *Bambusa arundinacea* roots.

CONCLUSION

The chemical examinations of the ethanol extract of *Bambusa arundinacea* roots yielded three compounds, 3', 3, 6, 7-tetramethoxy-4', 5, 8-trihydroxy flavone (HKS-04), 4-methoxy benzoic acid (HKS-05) and 4'-hydroxy flavan-3-ol (HKS-06) respectively on column chromatography and purification. All these compounds are characterized by spectral data and chemical tests.

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