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CHEMICAL INVESTIGATION OF THE BARK OF *ADENANTHERA PAVONINA LINN*

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ABSTRACT

The chemical investigation of the bark of *Adenanthera pavonina Linn.* have been found to contain the reducing sugar (1.01%) as glucose. The percentages of various amino acids present in the crude protein (5.25%) were found to be aspartic acid (0.10%), threonine (0.24%), serine (0.08%), glutamic acid (0.52%), glycine (0.09%), alanine (0.07%), valine (0.10%), methionine (0.13%), isoleucine (0.06%), tyrosine (0.27%), histidine (0.11%), lysine (0.88%) and arginine (0.25%). The fatty acid composition were found to be lauric (5.23%), palmitic (38.16%), oleic acid (6.29%) and stearic acid (8.93%).

Key words: *Adenanthera pavonina Linn*, Reducing sugar, Amino acids, Fatty Acids.

INTRODUCTION

The plant *Adenanthera pavonina Linn.* (Bengali Rakta Kambal) belongs to the family Leguminosae and is an important medicinal plant widely distributed in the Asian and African countries[1]. As an indigenous plant it is grown and cultivated mostly in the south-eastern region of Bangladesh[2]. Decoction of the seeds is used in pulmonary affections and externally applied in chronic ophthalmia. The seeds of the plant are used for the treatment of boils and inflammations[2,3]. Methanolic extract of the seeds and roots showed blood pressure lowering effect[2,4]. The leaves and the barks of the plant are used as a remedy for chronic rheumatism, gout, haematuria, haematemesis and diarrhoea[1-3]. The plant is also used as a cure for sore throat. The previous studies has demonstrated potent anti-inflammatory activity of its seeds and leaves[5,6]. Keeping in view the medicinal importance of the plant, it was thought worth while to undertake the chemical analysis of the extracts of the bark of *adenanthera pavonina Linn.*

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EXPERIMENTAL

Plant materials

The barks of *Adenanthera pavonina L.* were collected from a mature plant from Dhaka city of Bangladesh. A voucher specimen representing this collection (accession No.– DACB34196) was deposited in the National Herbarium of Bangladesh. The barks of *adenanthera pavonina L.* were shade-dried, pulverized by a mechanical grinder and passed through a 200 mesh sieve to obtain fine powder.

Analysis of reducing sugar

20 g of bark powder was refluxed with small quantity of calcium carbonate and 200 mL of distilled water for one hour. The aqueous extract was separated by decantation and the powder was further refluxed thrice with 100 mL of distilled water each time. The aqueous filtrates were combined and 10 percent solution of lead accetate was added till the precipitation was complete. It was filtered and the filtrate was neutralised with ammonia. This neutral solution was concentrated on a water bath till the volume became 100 mL.

Analysis of protein and amino acids

The bark powder of the *adenanthera pavonina* was used to determine the amount of protein and amino acids. Protein was determined by Kjeldhal Method[8].

Procedure for amino acid analysis[8]

The bark powder sample (4.0 g) was taken and a fine paste was made by mortar and pestle with 6 N HCl and then transferred in 250 mL round bottle flask placed in heating mantle at 110°C for 24 hours for hydrolysis of protein with 50 mL of 6 N HCl. After hydrolysis, the solution was kept in evaporating dish to evaporate HCl on water bath. The residue was again dissolved in 20 mL 0.1 N HCl and evaporation was repeated twice to remove excess of the acid. It was then filtered to 25 mL volumetric flask through Whatman No. 1 filter paper with 0.1 N HCl, known as stock solution. Again, the stock solution filtered through 0.45 μm syringe filter. Then the stock solution and standard solution were run through the amino acid analyzer[9].

Analysis of fatty acid

Fatty oil was collected by soxhlet extraction method with pet ether (40-60^{o}C) as solvent from bark powder of *adenanthera pavonina L.* It was a brownish yellow coloured oil.

Preparation of methyl esters of sample fatty acid fraction[8]

The pet ether extract oil of bark powder (150 mg) was taken in a pear shaped flask. Methanolic sodium hydroxide (0.5 M, 5 mL) was added to it and shaked well. The mixture was refluxed for 30 minutes in a boiling water bath. Then the mixture was evaporated to dryness by means of rotary evaporator. Boron-trifluoride methanol complex (BF_3-MeOH, 5 mL) was added to the dried material. The mixture was heated on a boiling water bath for 6-10 minutes. It was then evaporated to dryness and extracted with hexane (3 × 15 mL). The hexane soluble part containing the methylesters of fatty acid was analyzed by Gas Chromatography (GC). The methyl esters of authentic specimens were also injected to the GC (Shimadzu 9AM, Japan, Column-AT-5, FR-1 mL/min N_2, Detector-FID, Detector Temp. 290^0C, Injector Temp. 280^0C, Final Temp. Column 270^0C. Program: 140^0C–1 min–7^0/min–270^0C–10 min) under the same conditions.

RESULTS AND DISCUSSION

Identification of reducing sugar

For identification of sugars the spots of the concentrated test mixture and authentic samples (lactose, galactose, arabinose, glucuronic acid, maltose, glucose, xylose) were developed in an n-butanol : acetic acid : water (4 : 1 : 5 upper layer) solvent system[7] by running the chromatogram over night. After developing the chromatogram it was sprayed with silver nitrate reagent. The identity of test sugar have been confirmed by comparison of R_f value with authentic sugar (D-glucose) (Table 1). The amount of reducing sugar were estimated (glucose) by Fehling's method using methylene blue as indicator. Thus the percentage of reducing sugar as found to be present in the solution is 1.01 percent (glucose).

Table 1: **Analysis of reducing sugar**

Name of reducing sugar	R_f	
	Reported	**Observed**
D-glucose	0.18	0.19

Analysis of protein and amino acids

The protein content was found to be 5.25%, which is higher than the reported[7] protein content (4.5%) in the seeds.

Amino acid analyzer[9] showed the standard curve for standard solution and sample

curve for sample solution. By comparing the retention times[9], the amount of amino acids were calculated and the results are given in the Table 2.

Table 2: Amino acids in the bark of *Adenanthera pavonina L*

Amino acids	% of weight	Retention time (Sample)	Retention time (Standard)
Aspartic acid	0.10	8.776	8.081
Threonine	0.24	9.819	9.712
Serine	0.08	10.407	10.549
Glutamic acid	0.52	11.549	11.718
Glycine	0.09	16.332	16.026
Alanine	0.07	18.037	18.008
Valine	0.10	19.722	19.558
Methionine	0.13	21.892	22.066
Isoleucine	0.06	23.016	23.038
Tyrosine	0.27	27.093	26.668
Histidine	0.11	27.532	27.326
Lysine	0.88	32.088	32.052
Arginine	0.25	33.176	33.269

Analysis of fatty acid

Four prominent peaks of methyl esters were identified by comparing their retention times with the methyl esters of authentic specimen[10]. Thus the methyl esters of lauric (5.23%), palmitic (38.16%), oleic (6.29%) and stearic (8.93%) acids have been confirmed. (Fig. 1, 2 and Table 3). The other two major peaks could not be identified due to the lack of standard fatty acid esters.

Table 3: Analysis of fatty acid

Retention time	Name of fatty acid	Molecular formula (as methyl ester)	Area	Relative %
8.44	Lauric Acid	$C_{13}H_{26}O_2$	168	5.23

Cont…

Retention time	Name of fatty acid	Molecular formula (as methyl ester)	Area	Relative %
11.68	Palmitic Acid	$C_{17}H_{34}O_2$	1226	38.16
14.14	Oleic Acid	$C_{19}H_{36}O_2$	202	6.29
14.55	Stearic Acid	$C_{19}H_{38}O_2$	287	8.93
16.43	Not identified	-	612	19.05
20.76	Not identified	-	718	22.35

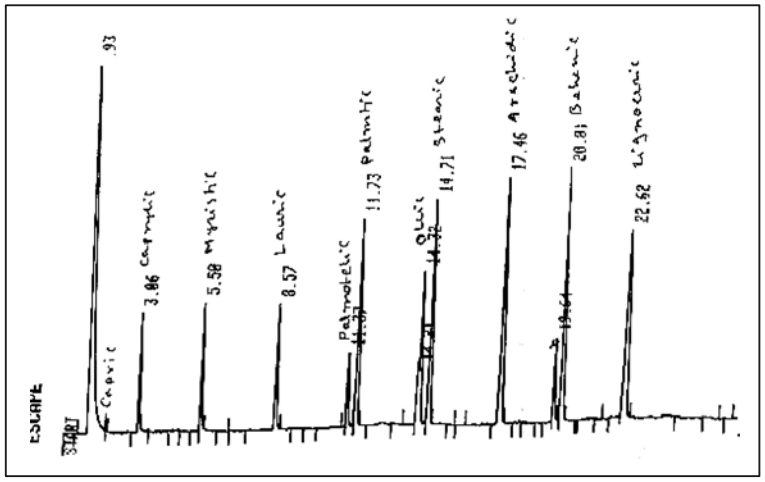

Fig. 1: GC spectrum of reference (standard) fatty acids

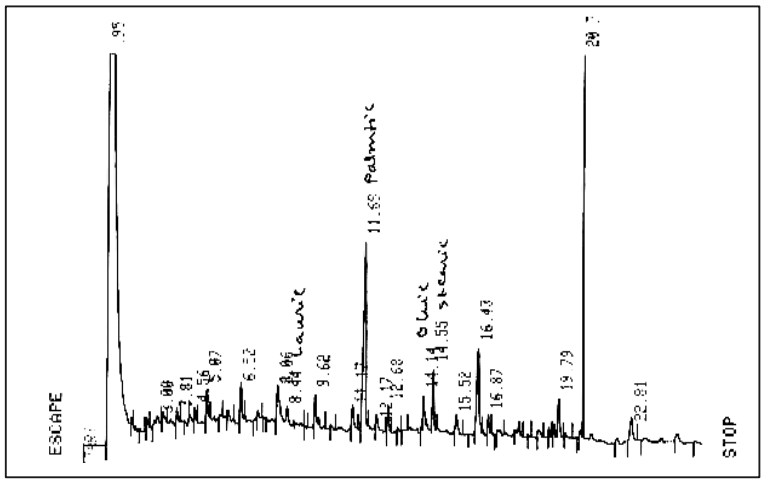

Fig. 2: GC spectrum of sample (pet. ether extractive) fatty acids

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REFERENCES

1. K. R. Kirtikar and B. D. Basu, Indian Medicinal Plants, Int. Book Distributors, 2nd Edn, Vol. **2**, Dehradun, India (1991) p. 908.
2. A Ghani, Medicinal Plants of Bangladesh (Chemical Constituents and Uses), Asiatic Society of Bangladesh, (2003).
3. WI (The Wealth of India-Raw Materials), CSIR New Delhi, India, IA (1985).
4. A. D. A. Adedapo, Y. O. Osude, A. A. Adedapo, J. O. Moody, A. S. Adeagbo, O. A. Olajide and J. M. Makinde, Blood Pressure Lowering Effect of Adenanthera Pavonina Seed Extract on Normotensive Rats, Records of Natural Prod., **3**, 82 (2009).
5. O. A. Olajide, C. A. Echianu, A. D. A. Adedapo and J. M. Makinde, Anti-inflammatory Studies on Adenanthera Pavonina Seed Extract, Inflammopharmacol, **12(2)**, 196 (2004).
6. C. Mayuren and R. Ilavarasan, Anti-inflamatory Activity of Ethanolic Leaf Extracts from *Adenanthera Pavonina (L)* in Rat, Pharmacognosy, **1(2)**, 125 (2009).
7. O. P. Chourasia and J. T. Rao, Chemical Investigation of the Seeds of *Adenanthera pavonina Linn*, Asian J. Chem., **18(4),** 3213 (2006).
8. A. Annonimus, Manual of Laboratory Techniques, National Institute of Nutrition, Indian Council of Medical Research, Hyderabad, India, (1976) p. 1.
9. Amino Acid Analyzer, Shimadzu Corporation, Japan; Detector: Fluorescent Detector, Model RF-10XL, Cat. No. 228-35353-38, Serial No C20953902790 YS, 220-230V/240V, 50- 60Hz, 350VA; Column Oven, Model CTO- 10 AVP; Flow Control, Model PRR- A; Auto Injector, Model SCL-10ADVP; Liquid Chromatograph, Model LC-10ADVP; A-D, Leak Model FCV- 10 ALVP; Degasser, Model DGU-14A.
10. O. P. Chourasia and J. T. Rao, Chemical and Anti-microbial Examination of the Fixed Oil from the Seeds of *Adenanthera Pavonina Linn*, Asian J. Chem., **17(1),** 289 (2005).

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