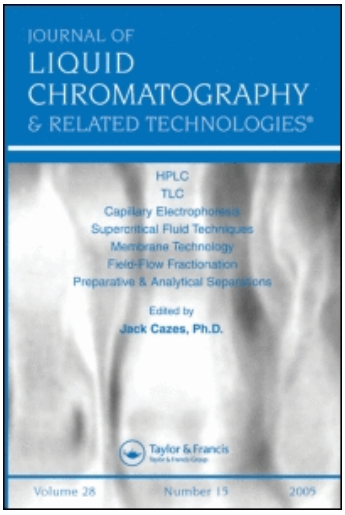

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Separation and Isolation of Limonoids from *Khaya Senegalensis* by Direct High Performance Liquid Chromatography

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SEPARATION AND ISOLATION OF LIMONOIDS FROM *KHAYA SENEGALENSIS* BY DIRECT HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

Isolation of various limonoids in pure form from the ethanol extract of the seeds of *Khaya senegalensis* (Desr.) A.Juss by Prep. HPLC procedure is described.

INTRODUCTION

The genus Khaya is a source of valuable timber, the African mahagony. Several species of this genus such as *K. grandifolia, K. anthotheca, K. ivorensis, K. senegalensis* etc. have been examined in great detail by Taylor and collaborators[1]. More than a dozen limonoids have been isolated from different species of this genus and from different parts of the species and structures assigned on the basis of spectral data and correlation studies.[2] All these compounds have been obtained by conventional column chromatography.

Experience in this laboratory has shown that preparative high performance liquid chromatography is exceedingly well suited for separation of tetranortriterpenoids as demonstrated in our studies on neem constituents.[3,4,5]

Table 1

Isolation of Various Limonoids from the Seeds of *K. senegalensis*

Peak	RT (min.)	Compound	Amount Obtained
1	20.57	mixture	70 mg
2	23.24	6-hydroxy methyl angoensate	50 mg
3	27.37	2-hydroxy mexicanolide - New	150 mg
4	38.71	3-deacetyl-7-keto khivorin	120 mg
5	45.21	3β-hydroxy mexicanolide (Δ14,15 instead of 8,14)	35 mg
		3β-hydroxy mexicanolide (Δ8,30 instead of 8, 14) - New	10 mg
6	50.60	2,3-dihydroxy mexicanolide - New	100 mg
7	58.44	mixture	40 mg
8	64.55	mixture	20 mg
9	74.43	3β-hydroxy mexicanolide	945 mg
10	94.22	3-deacetyl khivorin	640 mg
11	103.37	3,7-dideacetyl khivorin	610 mg
12	MeOH wash	mixture	3000 mg

Khaya senegalensis (Desr.) A. Juss has been introduced into India in the state of Maharashtra as part of an afforestration programme and fruits are available in the month of February. We wished to test the various constituents of seeds of this species for their antifeedant and antifungal activities. Column chromatography of the extract from this source is difficult since there are a large number of compounds of closely similar structure. Direct preparative HPLC is fast, reproducible *ad nauseam* and does not involve the hassle of examining a large number of fractions by TLC. Herein, we report the isolation of various compounds from the ethanol extract of seeds of *K. senegalensis*. Nine compounds were obtained, six of which are known and three new (Table 1). Materials from peaks 1,7,8 & 12 proved to be mixtures, which will be examined in greater detail for separation and isolation of other constituents in pure state by further chromatography.

MATERIALS AND METHODS

Preparative High Performance Liquid Chromatography was carried out using a Shimadzu LC 8A HPLC system linked to CR4A data processor and the

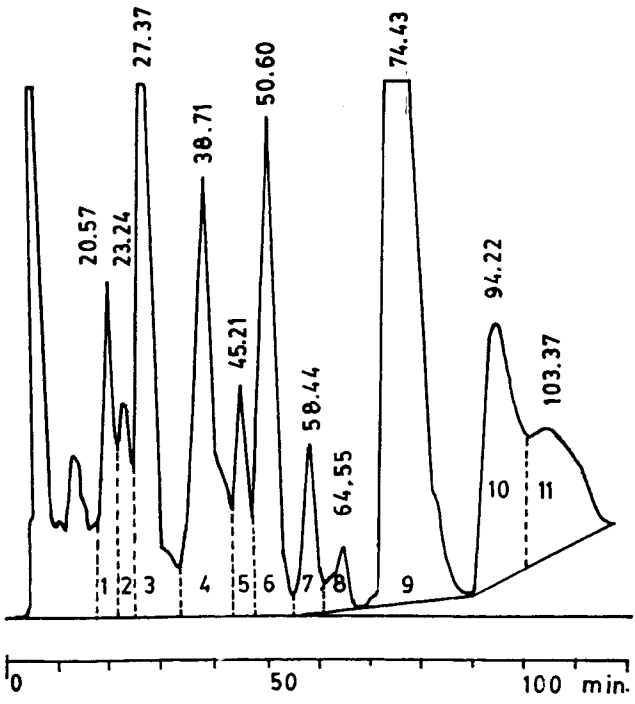

Figure 1. Preparative High Performance Liquid Chromatogram of the ethyl acetatefraction (VLC) from the seed extract of *Khaya senegalensis.*

peaks detected at 215 nm. Shimpack reverse phase (C_{18}) preparative column (25 cm x 20 mm i.d) was used for preparative runs and Phenomenex and Merck reverse phase columns (C_{18}) (25 cm x 4.6 mm) were used for analyses. Freshly powdered seeds (1.015 kgs) of *K.senegalensis* were extracted three times with n-hexane at room temperature and the defatted seed powder was extracted with ethanol.

The ethanol extract after removal of solvent (74.2 gm) was suspended in water and extracted with ethyl acetate. The residue (50.4 gm) was subjected to VLC using hexane and increasing quantities of ethyl acetate (10 - 50%) and then finally with ethyl acetate. The ethyl acetate fraction was concentrated to dryness in vacuo at 45° (10.5 gm). This was subjected to preparative HPLC for the isolation of the major limonoids.

RESULTS AND DISCUSSION

For each preparative run, 500 mg of the residue from ethyl acetate extract was dissolved in 2 mL of methanol, filtered through a Millipore filter (0.25 μm) and then injected into the preparative column (25 cm x 20 mm i.d.). The eluent flow rate was 10 mL / min. throughout the run. The individual peaks from peak 1 to 11 (Fig.1) were collected and evaporated. The purity of the compounds recovered from the peaks was checked by analytical HPLC. Identity of known compounds was established by spectral methods and comparison with literature data.

Peak 5 gave a mixture of two isomeric compounds which were separated by HPLC using ACN:MeOH:H_2O 35:30:35. In all three new compounds were obtained and their structures elucidated by spectral methods. Details will be published elsewhere.

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