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Isolation of a Cucurbitacin from *Picrorhiza Kurroa* by Column Chromatography and Its Characterization

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ABSTRACT

The roots and rhizomes of Picrorhiza kurroa Royle ex Benth. (Family-Scrophulariaceae) are used in traditional and modern medicines for liver disorders, fever, asthma, and jaundice. The commercial extract of Picrorhiza kurroa showed better yield and intense spots in HPTLC studies as compared to the methanolic extract of the rhizomes. The commercial extract showed the presence of 20 components when analyzed by a reverse phase HPLC method using gradient solvent system. A simple and convenient column chromatographic method was developed to isolate Cucurbitacin (aglycone part of Cucurbitacin glycoside) from the commercial extract and its structure was confirmed by chemical and spectral studies.

Keywords: Picrorhiza kurroa: Scrophulariaceae, rhizomes, HPLC, Cucurbitacin.

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INTRODUCTION

The alternative system of medicine is gaining importance worldwide and current systems such as Ayurveda are coming into focus. As per World Health Organization (WHO) data, health care needs of around 80 percent of the population of developing nations are fulfilled by traditional medicines.

For Global acceptance of herbal medicines, proper standardization techniques should be developed, which is the most important challenges faced by Ayurvedic drug industry. One of the important methods for standardization of herbal drugs is marker based standardization which helps in adjusting the herbal drug formulation to a defined content of constituent/s. A Marker compound may or may not have therapeutic activity.

Picrorhiza kurroa Royle ex Benth. (Family-Scrophulariaceae) is a small important alpine herb, having its habitat in the western Himalaya from Kashmir to Sikkim and growing at an altitude of 3000–5000 m. The roots and rhizomes of Picrorhiza kurroa are used in traditional and modern medicines for liver disorders, fever, asthma, and jaundice [1-4]. The plant is reported to possess hepatoprotective principles such as iridoid glycosides, antioxidant principles like apocynin and androsin, and antitumor principles like cucurbitacin glycosides [1, 5-9]. Literature survey gives number of isolation methods for picrosides and apocynin, but only one isolation method was reported for cucurbitacin glycosides which involve use of HPLC [10].

The present paper reports a simple and convenient isolation method for a Cucurbitacin from commercial extract of Picrorhiza kurroa and its characterization.

MATERIALS AND METHODS

The commercial extract of Picrorhiza kurroa was provided by Amsar Pvt. Ltd. Indore, India. The powdered rhizomes of Picrorhiza kurroa was obtained and authenticated from Piramal Life Sciences Ltd. Mumbai, India in December 2007.

Preparation of extracts:

The dried powdered rhizomes and commercial extract of Picrorhiza kurroa (10 gm) were extracted separately with methanol (150ml) for 12 hrs and filtered. The filtrate was evaporated on water bath and the extracts were compared for their yields and intensity of spots in HPTLC studies.

HPTLC/HPLC analysis:

The methanolic solutions of the extracts were analyzed by HPTLC using chloroform: methanol (82:18) as mobile phase. A reverse phase high pressure liquid

chromatography method was used to determine the number of components in the commercial extract of Picrorhiza kurroa. The commercial extract was dissolved in 50% aqueous methanol to give strength of 2.5 mg/ml and sonicated until dissolved. This solution was filtered through a 0.45 µm membrane filter and analyzed by a reverse phase high pressure liquid chromatography method using a gradient system consisting of acetonitrile and 1% acetic acid in varying proportion.

Isolation method:

The commercial extract (20gm) was dissolved in 250ml chloroform; the chloroform soluble portion (0.627 gm) was loaded on column made from Silica gel (60-120 mesh) and eluted with varying proportion of chloroform and ethyl acetate as eluting mixture. Fractions eluted with chloroform: ethyl acetate (70:30) gave a single spot at Rf 0.60 were pooled together which was named as Component I.

RESULTS AND DISCUSSION

The yield of commercial extract (68.96%w/w) was higher as compared to the rhizome extract prepared in the laboratory (44.80%w/w) (Table no 1). Both the methanolic extracts were compared for number of spots and the intensity of peaks by HPTLC analysis. The plates were observed under UV light at 254nm, 365nm and 540nm. No spots were seen under 365nm and 540nm. Seven spots were observed at 254nm for both the extracts. The Rf values of these spots were same for both the extracts, commercial extract showed intense spots and hence, was used for further studies. The commercial extract showed seven spots on TLC plate with the given mobile phase indicating 7 constituents with Rf value 0.21, 0.32, 0.44, 0.61, 0.73, 0.78, 0.83 (Table no. 2). The HPTLC plates were sprayed with Anisaldehyde Sulphuric acid Reagent (ASR) to detect the presence of various constituents such as phenols, steroids, terpenes and bitter principles. The commercial extract gave yellow and grey zones (at day light) with ASR indicating the presence of iridoid glycosides, phenols and Cucurbitacin glycosides (Figure no. 1) [11].

Table no. 1-Comparison of commercial extract and rhizome extract

Sr. No.	Parameters	Commercial extract		Rhizome extract
1	Colour	Brown		Brown
2	pH	5.85		6.05
3	Yield	**After 4 hrs**	**After 12 hrs**	**After 12 hrs**
		62.5%w/w	68.96%w/w	44.80%w/w

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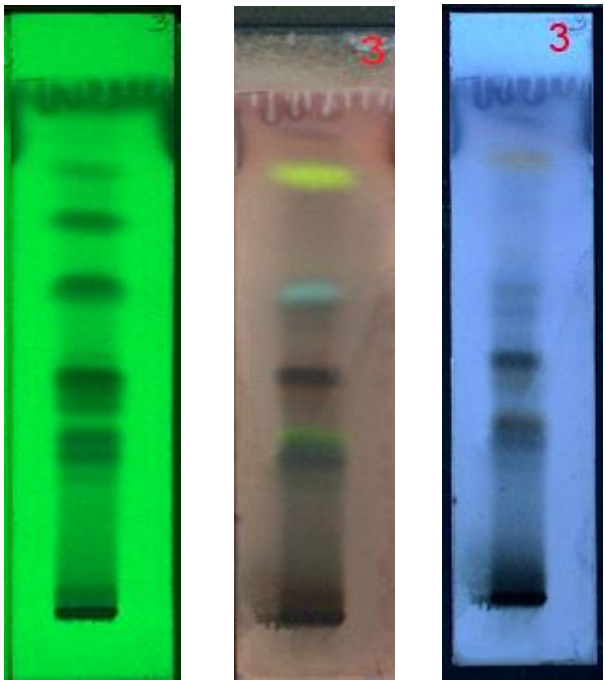
Table no. 2-HPTLC analysis of commercial extract

Visualization	at 254nm	at 365 nm	After spraying with ASR
No. of spots Rf	7 0.21, 0.32, 0.44, 0.61, 0.73, 0.78, 0.83	4 0.32, 0.73, 0.60, 0.83	6 0.33, 0.46, 0.60, 0.73, 0.85



at 254 nm at 366nm at 540 nm
With ASR

Figure no. 1-Video-images of TLC plates of commercial extract

The HPTLC analysis of commercial extract revealed 7 constituents with the given mobile phase. The extract was also analyzed by HPLC to detect the number of constituents present in the extract. The λ_{max} of the commercial extract was found to be 265-270nm; hence 265nm was used for HPLC analysis. HPLC profile of commercial extract gave 20 peaks at retention times 13.950, 28.892, 29.558, 30.525, 31.325, 31.767, 32.725, 34.133, 35.050, 36.467, 37.542, 39.000, 42.092, 44.058, 45.708, 49.175, 51.542, 56.292, 57.750 and 58.917 min (Figure no. 2).

The yield of the component I, isolated from commercial extract, was obtained as 13.6mg w/w of the commercial extract. It was obtained as an amorphous white powder, melting point 95-97ºC and had Rf 0.6 with mobile phase toluene: ethyl acetate (6:4). The elemental analysis confirmed the absence of nitrogen and halogens in component I.

All Cucurbitacins are seen with Vanillin Phosphoric acid reagent (VP) as weak yellow-brown and blue violet zones (at day light). Cucurbitacin also show characteristic bright yellow-green and red fluorescence with Vanillin-Sulphuric acid reagent (VS) in UV-365nm. The

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Cucurbitacin glycosides migrate preferably to Rf value 0.10-0.40, the aglycones to Rf value 0.50-0.90 with mobile phase chloroform: methanol (95:10) [12].

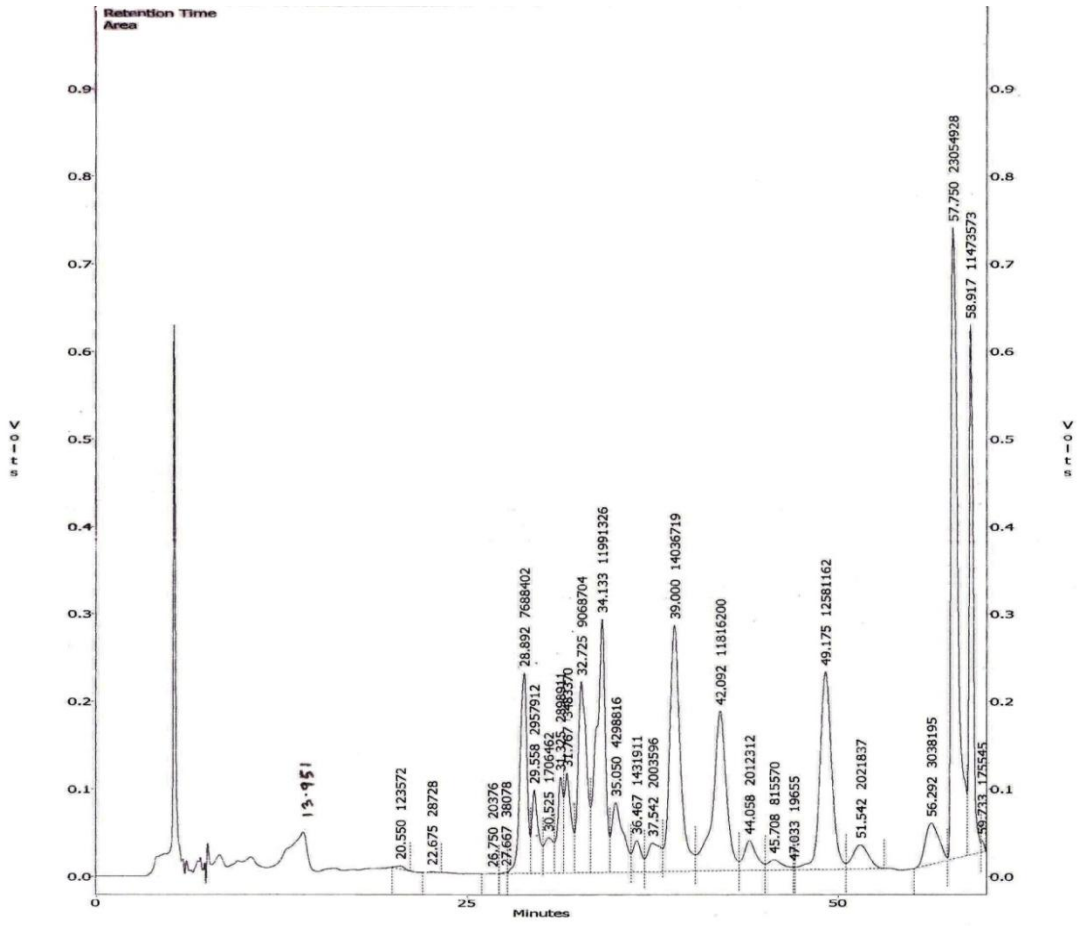

Figure no. 2-HPLC fingerprint of commercial extract

Component I gave yellow colour with ASR indicating presence of bitter principles like cucurbitacin or terpenic (Cucurbitacin is triterpenic in nature) compound (Figure no.3). Component I showed orange brown zone (at day light) with VP and yellow-green fluorescence in UV-365nm with VSR indicating presence of Cucurbitacin (Figure no. 4). Component I gave a single peak at Rf 0.86 with mobile phase, chloroform: methanol (95:10) (Figure no. 5).

Component I : **UV max** (methanol): 220, 275 nm; **IR bands** (KBr): 3312.07, 2926.28, 1662.79, 1602.99, 1577.91, 1512.33, 848.76, 815.96, 669.30 cm^{-1}; **^{1}H-NMR** (400 MHz, DMSO): δ: 0.832 (3H, s), 1.212 (3H, s), 2.45 (1H, d), 3.331 (1H, s), 10.325 (1H, d) hydroxyl hydrogen next to carbonyl group; **^{13}C-NMR** (300 MHz, DMSO): 26.256 (- CH_3), 54.914 (C-OH), 70.87 (C-2), 89.811 (C-22), 115.295 (C-6), 128.563 (C-24), 130.701 (C-25), 162.013 (C-3), 196.030 (C-11). The

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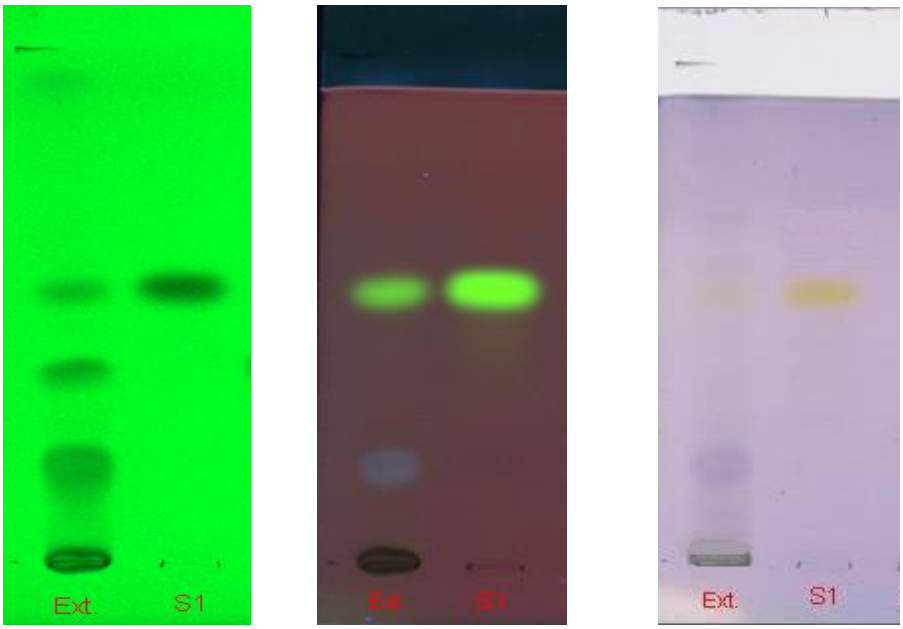
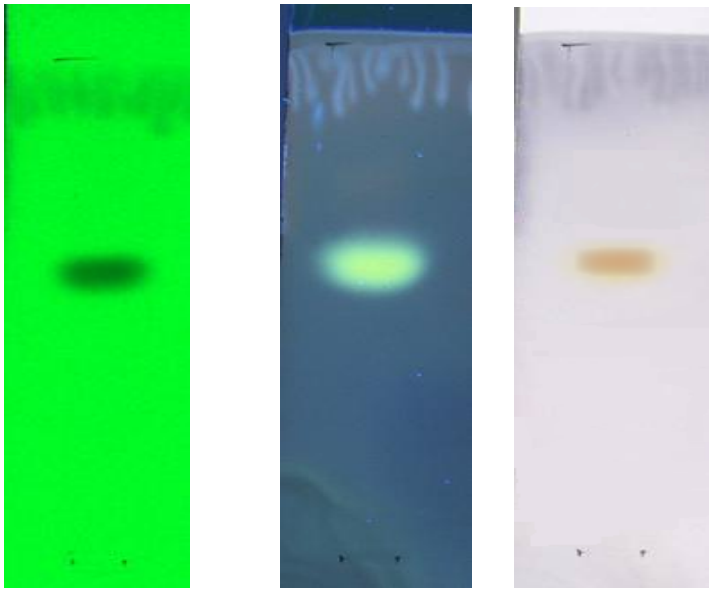
component I was identified by comparing the ^{1}HNMR and 13CNMR data with that reported in literature [10, 13].



at 254nm at 366nm at 540nm. Derivatization with ASR

Figure no. 3-Video-images of TLC plates showing chloroform extract and component I



at 254nm at 365nm at 540nm. With VP reagent

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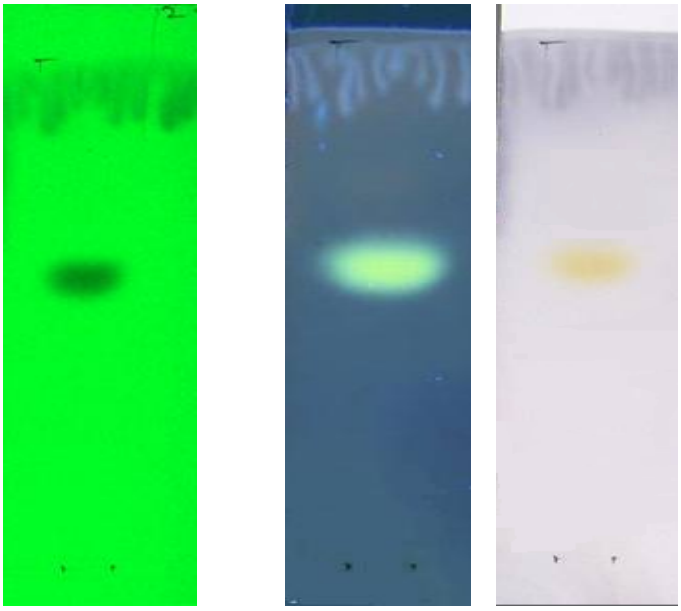

at 254nm at 365nm at 540nm
With VS reagent

Figure no. 4-Video-images of TLC plates showing component I

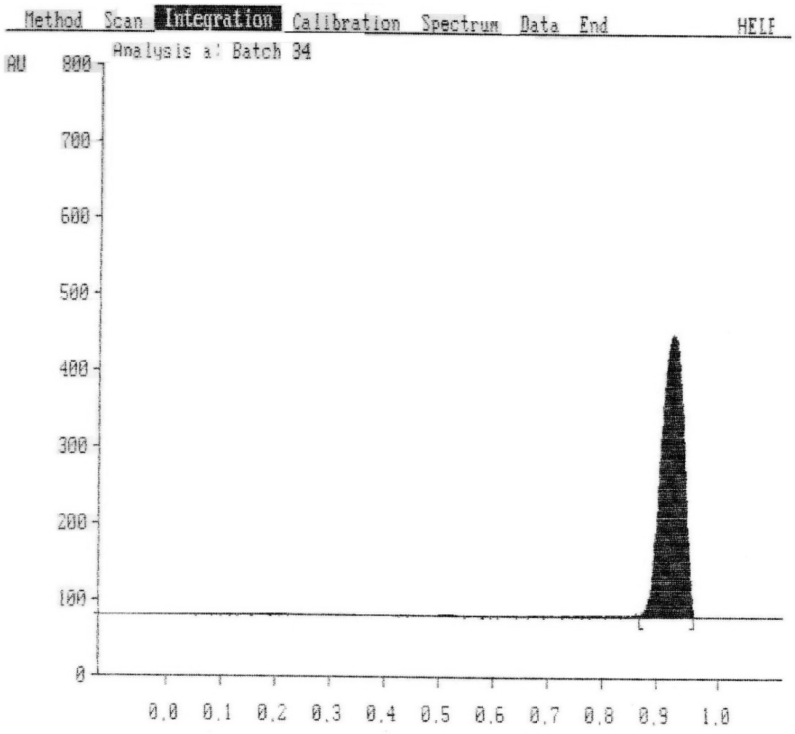

Figure no. 5-HPTLC fingerprint of component I (Rf 0.86) with mobile phase chloroform: methanol.

Thus, from spectral and chemicals studies structure of component I was confirmed as Cucurbitacin (Figure no. 6).

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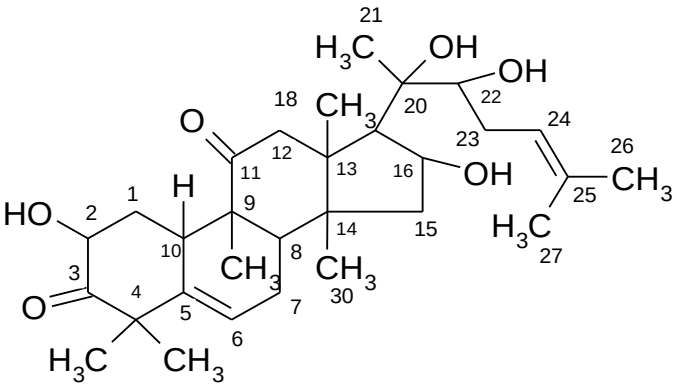

Figure no. 6 structure of cucurbitacin

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