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Research Article

CHROMATOGRAPHIC SEPARATION OF BIOACTIVE COMPOUNDS FROM *IPOMOEA BATATAS LAM* (SWEET POTATOES) BY COLUMN, HIGH-PERFORMANCE THIN LAYER CHROMATOGRAPHY, AND GAS CHROMATOGRAPHY-MASS SPECTRUM ANALYSIS TECHNIQUES

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ABSTRACT

Objective: The plant kingdom has been used for health and medicinal purposes for several thousands of years. The usage of plants to treat a variety of different ailments is universal and exists in every human culture on earth. Every plant on the planet creates specific chemical compound which is a basic part of their metabolic function. In the present study, Ipomoea batatas is targeted which is regularly used in diet, to identify its potential therapeutic property.

Methods: To discover new bioactive compounds from plant sources, which can be used as new lead molecules, the Ipomoea batatas extracts was to be subjected to various chemical screening techniques such as Column Chromatography, high-performance thin layer chromatography (HTLC), gas chromatography-mass spectrometry (GC-MS), which quickly provides plenty of structural information, resulting in partial or complete structure determination of natural products.

Results: Totally 75 fractions were collected from column chromatography and they were pooled to five fractions according to their polarity and TLC patterns, and were subjected to MTT assay. Out of which, the active fraction 3 (ethylacetate:ethanolic fraction) showed increased cytotoxicty on HepG2 cells (liver cancer cells) at IC50 (µg/mL) as 33.52, compared to rest of the four active fractions of I. batatas. The chromatogram of HPTLC of active Fraction 3 (ethylacetate:ethanolic) extract of I. batatas showed four spots at the following Rf 0.45, 0.49, 0.85, 0.91 and were found to be more predominant as their area was 70.9, 315.5, 90.6, 125.0. GC-MS gave 44 compounds out of which more than 20 compounds showed medicinal property.

Conclusion: It has been found that the pharmacological property shown by the active Fraction 3 (ethylacetate:ethanolic fraction) of I. batatas might be due to single compound or due to the cumulative effect of all the compounds in composite

Keywords: Ipomoea batatas, Gas chromatography-mass spectrometry, High-performance thin layer chromatography.

INTRODUCTION

Plants are used as medicines in all countries and are the source of powerful drugs [1]. The use of specific plants and their application methods for particular ailments were passed down through oral history. As the information about the plants were recorded in herbals [2,3]. The plant kingdom is an open reservoir of many molecules with potential therapeutic property. Only a small percentage of known plant species have been studied from a phytochemical or a pharmacological point of view [4]. Plants have been traditionally proved to be a rich source of novel drug compounds, and the herbal mixtures have given a great contribution to human health and well-being. The world health organization has recommended evaluating the effective plants to be used as safe, modern drugs. Therefore, the search for drugs and dietary supplements derived from plants has been started in recent years [5]. Some of these plants produce valuable drugs, which have high export potential [6]. Traditionally different parts of several medicinal plants or their extracts are used in the treatment of various diseases in India [7]. To discover new bioactive compounds from plant sources, which can be used as new lead molecules, the plant extracts has to be subjected to various chemical screening techniques such as high-performance thin layer chromatography (TLC), gas chromatography-mass spectrometry (GC-MS), which quickly provides plenty of structural information, resulting in partial or complete structure determination of natural products [8]. In the present study, one such plant is targeted which is regularly used in diet without knowing its potential therapeutic property.

METHODS

Collection and authentication of plant material

The white Ipomoea batatas was obtained from plant cultivator. It was authenticated at the National Institute of Herbal Science, Plant Anatomy Research Center, Chennai as the tubers of sweet potatoes and was confirmed as I. batatas lam, under the family Convolvulaceae.

Preparation of the extract

Fresh I. batatas, was washed under running tap water, air-dried, and then homogenized to fine a powder and stored in airtight bottles. About 30 g of powder was added to 90 ml of solvent (double distilled water, 1:3 ratios) in a dry flask. The flask was then incubated for 24 hrs in a shaker. After incubation, the extract was collected using Whatman No. 1 filter paper and evaporated below 40°C, which was used for further phytochemical analysis. Crude aqueous extract of I. batatas, was subjected to column chromatography.

Column chromatography

The extract was subjected to column chromatography using different solvent systems. The fractions collected were pooled over the polarity of the eluted solvent. Silica gel (100-200 mesh) was used as stationary phase. Column chromatography was done using a glass column. The dimension of the column was 15 cm × 4 cm. The column was packed with silica gel by wet packing method where a padding of cotton was placed at the bottom of the column. Slurry was made with the required amount of stationary phase (silica gel) in the solvent of slowest polarity (n-hexane) and poured into the column to form a

bed of silica. The extract was made into admixture (1:3 ratio) of silica gel (100-200 mesh) then poured on to the top of silica gel making a slurry, a layer of cotton was covered again and allowed to percolate. The column was then eluted gradiently with solvents of increasing polarity. The fractions were collected, and the solvent was recovered by simple distillation. All the concentrated fractions were subjected to TLC, and similar fractions were combined. Totally 5 fractions were obtained after pooling based on TLC patterns.

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay for cell viability

The MTT assay [9] is based on the ability of live but not dead cells to reduce a yellow tetrazolium dye to a purple Formazan product. This assay was performed on human liver cancer cell lines (human hepatocellular liver carcinoma cell line [HepG2]). The cells were plated in 96 well flat bottom tissue culture plates at a density of approximately 1.2 × 104 cells/well and allowed to attach overnight at 37°C. The medium was then discarded, and cells were incubated with different concentrations of all the fractions for 24 hrs. After the incubation, medium was discarded, and 100 µl fresh medium was added with 10 µl of MTT (5 mg/ml). After 4 hrs, the medium was discarded, and 100 µl of dimethyl sulfoxide was added to dissolve the Formazan crystals. Then, then absorbance was read at 570 nm in a microtiter plate reader. Cell survival was calculated by the following formula:

Viability % = (Test OD/Control OD) × 100
Cytotoxicity % = 100 – Viability %

High performance thin layer chromatography (HPTLC) fingerprint profile of *I. batatas*

Instrumentation

A Camag HPTLC system (Muttenz, Switzerland) equipped with a sample applicator Linomat V, twin trough plate development chamber, TLC Scanner 2, CATS Software and Hamilton (Reno, Nevada, USA) syringe (100 µl).

Chromatographic conditions

Chromatography was performed using HPTLC silica gel 60 F_{254} precoated plates (10 cm × 10 cm) (E. Merk Ltd., Darmsladt, Germany), 10 µl of the sample was applied on the plates as bands of 10 mm width with the help of (Hmilton Microsyringe, Switzerland), mounted on a Linomat V applicator, at the distance of 15 mm from the edge of the plates. The mobile phase constituted of (chloroform:methanol:formic acid-7:3:1, v/v/v). The plates were developed up to a distance of 85 mm (distance to the lower edged was 10 mm) was performed at room temperature in a camag twin trough chamber, previously equilibrated with mobile phase for 30 minutes.

The chromatographic conditions have previously been optimized to achieve the best resolution peak shape. After development, plates were dried undercurrent of air at room temperature. After drying the plates were dipped in vanillin sulfuric acid reagent and heated in air oven at 105°C, until the color of the spots appeared. Densitometry evaluation of the plates was then performed with Camag TLC scanner 2 equipped with CATS Software version 3.20 at max 260 nm. The chromatogram was recorded.

Table 1: *I. batatas* percentage yield of different fractions (total weight of extract-50 g)

Fraction number	Solvent	Obtained weight of fractions	Percentage of yield
Fraction 1	Hexane:ethyl acetate	2000 mg	4.0
Fraction 2	Ethyl acetate, ethylacetate:ethanol	4300 mg	8.6
Fraction 3	Ethyl acetate:ethanol	5700 mg	11.4
Fraction 4	Methanol	6500 mg	13
Fraction 5	Distilled water	20,000 mg	40

I. batatas: Ipomoea batatas

Determination of bioactive constituents of the active fractions

GC-MS analysis

GC-MS technique was used in this study to identify the phyto components present in I. batatas active Fraction 1. GC-MS analysis of I. batatas extracts was performed using a Perkin-Elmer GC Clarus 680 system and gas chromatograph interfaced to a mass spectrometer (clarus 600) equipped with an Elite-5 MS, fused silica capillary column (30 mm × 0.25 mm 1D × 250 µm df, composed of 100% dimethyl polysiloxane). For GC-MS detection, an electron ionization system with ionizing energy of 70 eV was used. Helium gas (99.999%) was used as the carrier gas at constant flow rate of 1 ml/minutes and an injection volume of 1 µl was employed (split ratio of 10:1); injector temperature 250°C; Ion-source temperature 230°C. The oven temperature was programmed

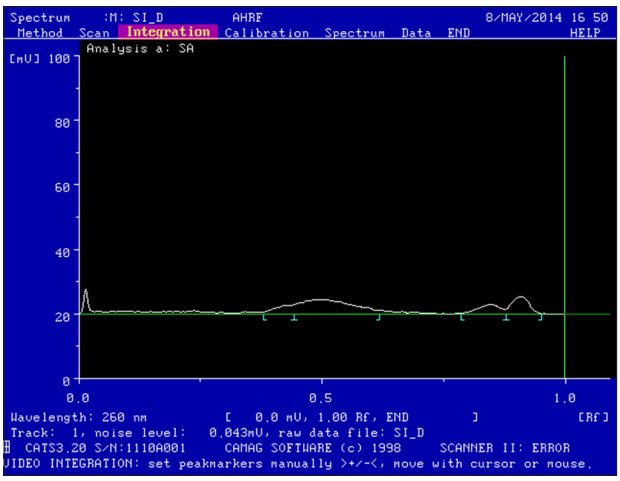

Fig. 1: High-performance thin layer chromatography chromatogram for sample A chromatogram of active fraction 3 (ethyl acetate:ethanol) of *Ipomoea batatas* extract

Table 2a: Anticancer effect of *I. batatas* on human liver cancer cell lines (Hep-G2)

S. No	Concentration (µg/mL)	I	II	III	IV	V
1	1000	16.77±0.60	36.49±0.55	23.45±0.60	6.60±0.66	12.34±0.58
2	500	32.10±0.98	41.16±0.57	29.47±0.51	15.52±0.58	16.64±0.67
3	250	45.22±0.32	44.44±0.66	31.84±0.41	19.93±0.68	21.24±1.01
4	125	56.25±0.36	53.00±0.76	45.56±0.57	27.31±1.30	33.81±0.96
5	62.5	64.57±0.76	57.13±1.27	48.64±0.67	34.14±1.31	38.99±0.33
6	31.25	71.90±00.44	63.34±0.66	51.22±0.59	53.43±1.63	60.76±0.81
7	15.625	79.61±0.37	66.81±0.58	56.18±0.28	63.45±0.60	67.16±1.26
8	7.8125	83.37±0.63	74.56±0.55	64.45±0.66	70.71±0.79	73.43±0.66
9	Cell control	100	100	100	100	100

I. batatas: Ipomoea batatas, Hep-G2: Human hepatocellular liver carcinoma cell line

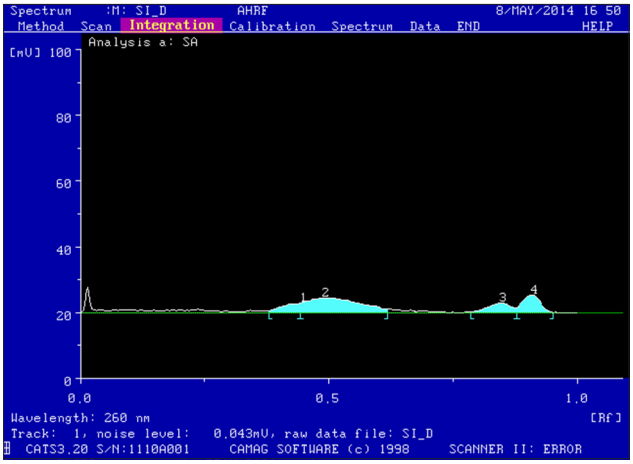

Fig. 2: High performance thin layer chromatography chromatogram for sample A of active fraction 3 (ethyl acetate:ethanol) of *Ipomoea batatas* extract

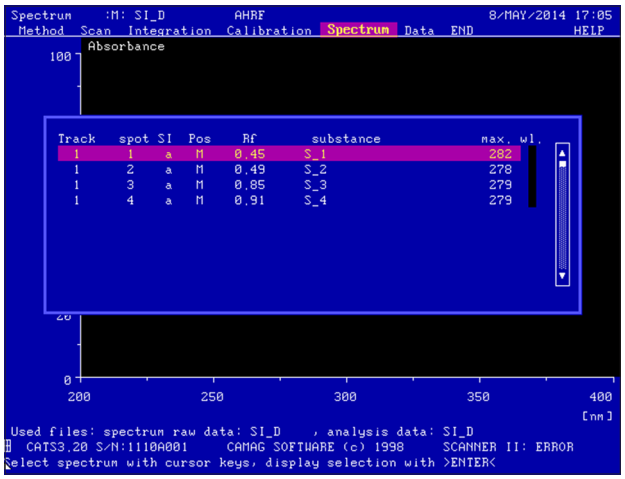

Fig. 3: Spectrum of high-performance thin layer chromatography for sample A of active fraction 3 (ethyl acetate:ethanol) of *Ipomoea batatas* extract

Table 2b: IC-50 concentration of samples

S. No	Samples	IC_{50} (µg/mL)
1	Active fraction-1	178.52
2	Active fraction-2	139.62
3	Active fraction-3	33.52
4	Active fraction-4	35.52
5	Active fraction-5	40.52

IC_{50}: Inhibitory concentration

from 60°C (isothermal for 2 minutes), with an increase of 10°C/minutes to 200°C, then 250°C/minutes to 30°C, ending with a 6 minutes isothermal at 300°C. Mass spectra were taken at 70 eV; a scan interval of 0.5 seconds and fragments from 50 to 600 Da. Solvent delay = 2.00 minutes, transfer temperature = 230°C, source temperature = 230°C, total GC running time was 32 minutes. The relative % amount of each component was calculated by comparing

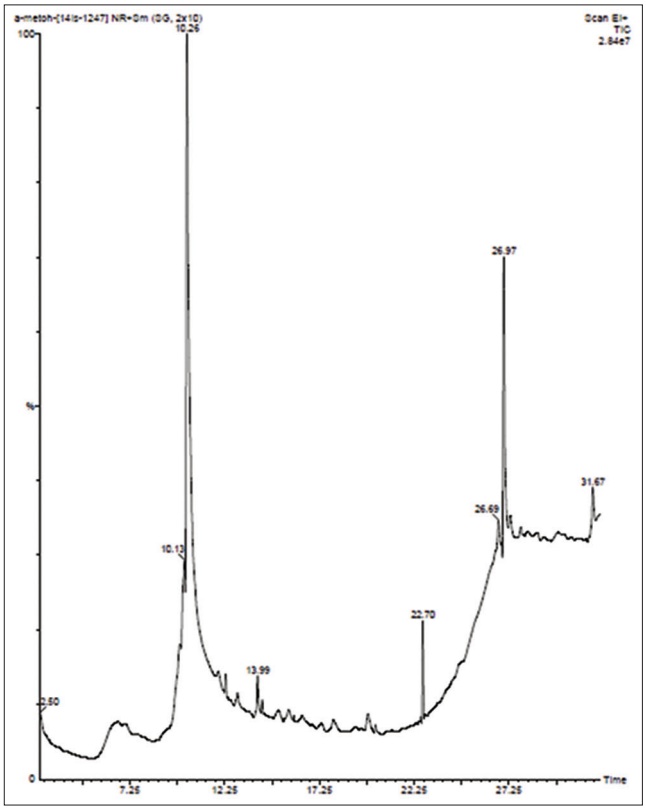

Fig. 4: Gas chromatography-mass spectrum analysis chromatogram of active fraction 3 (ethyl acetate:ethanol) of *Ipomoea batatas* extract

Table 3: Peak list and Rf value of the chromatogram of 10 µl of active fraction 3 (ethyl acetate:ethanol) of *I. batatas* extract

Peak	Rf	Height	Area	Max
1	0.45	3.0	70.9	282
2	0.49	4.5	315.5	278
3	0.85	3.0	90.6	279
4	0.91	5.4	125.0	279

I. batatas: Ipomoea batatas

Table 4: Compounds present in an aqueous extract of *I. batatas* (active fraction 3, ethyl acetate:ethanol)

Peak	Rev	Compound name	Molecular weight	Molecular formula	Activity
1	730	3',8,8'-Trimethoxy-3-Piperidyl-2,2'-Binaphthalene-1,1'4,4'-Tetrone	487	$C_{28}H_{25}O_7N$	Antioxidant
2	74	Phthalic acid, 2-ethylhexyl pentadecyl ester	488	$C_{31}H_{52}O_4$	Antidysentrica, antimicrobial
3	813	1,2-benzenedicarboxylic acid, diisooctyl ester	390	$C_{24}H_{38}O_4$	Anticancer, antiarthritic, anti-inflammatory
4	744	Phthalic acid, 2-ethylhexyl undecyl ester	432	$C_{27}H_{44}O_4$	Anti-peroxisome proliferation
5	764	3-oxo-18-nor-ent-ros-4-ene-15. beta.,16-acetonide	346	$C_{22}H_{34}O_3$	Antifeedent, antiviral
6	766	1,2-benzenedicarboxylic acid, mono (2-ethylhexyl) ester	278	$C_{16}H_{22}O_4$	Antioxidant, anti-inflammatory
7	831	1,2-benzenedicarboxylic acid, diisooctyl ester	390	$C_{24}H_{38}O_4$	Antimicrobial, antifouling

(Contd...)

Table 4: (*Continued*)

Peak	Rev	Compound name	Molecular weight	Molecular formula	Activity
8	800	Bis (2-ethylhexyl) phthalate	390	$C_{24}H_{38}O_4$	Antimicrobial, cytotoxic activity
9	821	di-n-octyl phthalate	390	$C_{24}H_{38}O_4$	Anti-venom activity
10	787	5-(hydroxymethyl)-2-(dimethoxymethyl) furan	172	$C_8H_{12}O_4$	Antioxidant
11	638	5-methyl-thiophene-2-carboxamide	141	C_6H_7ONS	Antibacterial, antifungal
12	612	2-oxabicyclo [3.1.0] hex-3-ene-6-carboxylic acid, ethyl ester	154	$C_8H_{10}O_3$	Not known
13	605	2-furancarboxaldehyde, 5-(hydroxymethyl)-	126	$C_6H_6O_3$	Antimicrobial, preservative
14	604	N-isobutyl-2(e),6(z),8(e)-decatrienamide	221	$C_{14}H_{23}ON$	Anti-inflammatory, analgesic activity
15	550	4-mercaptophenol	126	C_6H_6OS	Cytotoxicity
16	558	5-acetoxymethyl-2-furaldehyde	168	$C_8H_8O_4$	Anticancer, taste modulator
17	556	1-ethyl-3-methylcyclohexane (c, t)	126	C_9H_{18}	Toxic, harmful
18	541	Methyl 5,9-heptacosadienoate	420	$C_{28}H_{52}O_2$	Not known
19	526	Cyclohexane, 1-ethyl-4-methyl-, cis-	126	C_9H_{18}	Antimicrobial, preservative
20	514	2-thiophenecarboxylic acid, 5-methyl-	142	$C_6H_6O_2S$	Antibacterial, antifungal
21	500	Dimethyl 3-methylglutaconate	172	$C_8H_{12}O_4$	Antineoplastic activity

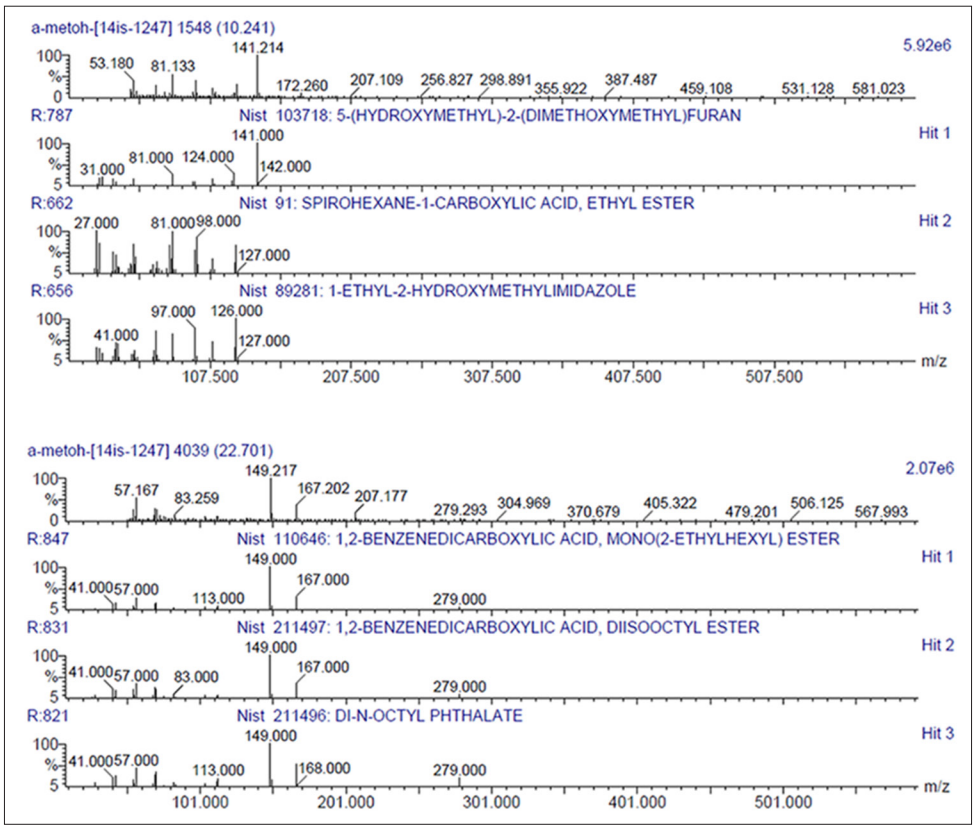

Fig. 5: Mass spectra of the various compounds present in active fraction 3 (ethyl acetate:ethanol) of *Ipomoea batatas* extract

its average peak area to the total areas, software adopted to handle mass spectra and chromatograms was a GC-MS solution ver. 2.53.

Identification of components

Interpretation of the mass spectrum GC-MS was done using the database of National Institute Standard and Technique (NIST08s), WILEY8 having more patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST08s, WILEY8 library. The name, molecular weight, molecular formula, and structure of the component were ascertained.

RESULTS AND DISCUSSION

The initial study was carried out with column chromatography for isolation of active fractions. Totally 5 fractions were collected from column and they were pooled to five fractions according to their polarity and TLC patterns, the details of collected fractions were given in the (Table 1), all the five active fractions obtained were subjected to MTT assay (Table 2a and b) cell viability and cell toxicity assay on HepG2 cells lines, the active fraction 3 (ethylacetate:ethanolic fraction) showed increased cytotoxicty on HepG2 cells (liver cancer cells) at IC_{50} (µg/mL) as 33.52, compared to rest of the four active fractions of I. batatas. The chromatogram

shown in (Figs. 1-3) of HPTLC indicates that the sample constituents were clearly separated without any tailing and diffuseness. It is evident from the (Table 3) that in 10 µl of active Fraction 3 (ethylacetate:ethanolic) extract of I. batatas there are four spots at the following Rf 0.45, 0.49, 0.85, 0.91 and were found to be more predominant as their area was 70.9, 315.5, 90.6, 125.0, respectively. GC-MS gave 44 compounds out of which more than 20 compounds showed medicinal property (Figs. 4 and 5, Table 4).

CONCLUSION

It has been found that of active Fraction 3 (ethylacetate:ethanolic fraction) contains not a single compound but a mixture of compounds, so the paharmacological property shown by the active Fraction 3 (ethylacetate:ethanolic fraction) of I. batatas might be due to single compound or due to the cumulative effect of all the compounds in composite.

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