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High Performance Liquid Chromatographic (HPLC) Analysis A Herbal Formulation *ACTP*

S.Selvakumar* and R. Valliammai

Department of Industrial Biotechnology,Bharath university, Chennai-6000073, India.

Abstract: Traditional knowledge of medicinal plants has always guided the search for new cures. In spite of the advent of modern high throughput drug discovery and screening techniques, traditional knowledge systems have given clues to the discovery of valuable drugs.Traditional herbal medicinal plants are often cheaper, locally available and easily consumable, raw or as simple medicinal preparations. presently, traditional medicinal practices form an integral part of complementary or alternative medicine. These simple medicinal preparations often mediate beneficial responses due to their active chemical constituents. Natural products have been the starting point for the discovery of many important hidden drugs. This fact has led to chemical and pharmacological investigations and general biological screening programs for natural products all over the world. Hence, it is of interest to investigate the phytochemical constituents of a poly herbal formulation ACTP. The present sudy aims at quantifying the phyto constituents by chromatographic methods. Therefore, it is of interest to investigate the HPLC analysis of the chloroformic extract of the herbal formulation ACTP were studied. Our results indicate that the presence and purity of 28 phyto compounds.
Key words : Cancer , Chemoprevention , Drug discovery , Phyto constituents , HPLC.

1 . Introduction

Cancer is a growing health problem around the world particularly with the steady rise in life expectancy, increasing urbanization and the subsequent changes in environmental conditions, including lifestyle. According to a recent report by the World Health Organization, there are now more than 15 million cases of cancer per year worldwide and there is no magic bullet that can completely conquer cancer, many types of the disease might be avoidable. Cancer risk can be reduced by eliminating the identified carcinogens or at least minimizing exposure to them but, without complete identification of the corresponding risk factors, such primary prevention might be difficult to implement. Furthermore, the avoidance of some risk factors could require large lifestyle changes, which are not easy to implement[1]. Many dietary constituents can increase the risk of developing cancer, but there is also accumulating evidence from population as well as laboratory studies to support an inverse relationship between regular consumption of fruit and vegetables can reduce the risk of specific cancers. Many clinical trials on the use of nutritional supplements and modified diets to prevent cancer are ongoing. It is conceivable that in the future people might only need to take specially formulated pills that contain substances derived from edible plants to prevent cancer or delay its onset. However, a precise assessment of the mechanisms by which the components of fruit and vegetables prevent cancer is necessary before they can be recommended for inclusion in dietary supplements or before they can be tested in human intervention trials [2].

2. Materials and Methods

2.1. Plant Materials

The herbal formulation *ACTP* was prepared in the department of Industrial Biotechnology, Bharath Institute of Higher Education and Research, Bharath University, Chennai, India. The equal volume of shade dried leaves of *Acalypha indica, Couropita guinensis, Tritax brogumbenz and Plectra amdoilicus* were taken in to marter and pistle,The plant material was coarsely powdered, then filtered by muslin cloth and the filtrate was used for further extraction.

2.2. Preparation of Plant extract

500 g of the herbal powder *ACTP* were charged in an aspiration bottle and allowed to soak in chloroform for 96 hrs at room temperature. The extract was filtered and concentrated on a water bath. The inorganic material was precipitated and filtered off. The filtrate were again concentrated in a China dish and dried in vacuum. The yield of the extract were weighed and will be stored in refrigerator for further use.

2.3. Chemicals and Reagents

All chemicals were used for this project were purchased from M/s. Sigma Chemicals, USA.

2.4. High performance liquid chromatography (HPLC) analysis of ACTP.

Analysis of chloroformic extract of *ACTP* was performed by HPLC. The HPLC system consists of LC-20AT prominence liquid chromatograph pump and SPD-20A prominence UV-Vis detector and Rheodyne type injector fitted with 20 L capacity fixed loop all from Shimadzu Corporation, Japan. The column used was Phenomenexluna 5 C18 (2) 100A (250 mm 4.6 mm) at ambient temperature. The output signals were monitored and processed using spinchrom CFR software. The solvent system optimized for the analysis was methanol: acetonitrile: water in the ratio 25:35:40. The flow rate was 1 mL/minute and detection wave length was set at 232 nm. The run time of the method was 10 minute and all analytes were separated within the run time [3].

3. Results and Discussion

HPLC analysis of the herbal formulation ACTP.

HPLC-Required Chromatographic Conditions

- Project code : 18A
- Test item name : ACTP-S1
- Analysis type : HPLC Profile

- Instrument Name : HPLC-LC-2010 SHIMADZU
- Stationary phase : C18-250×4.6 -5µ 100 A 0 (waters-Reliant)
- Mobil phase : Pump-A-Acetonitril: B-0.01% TFA (Time/%B)0.01/95, 12.0/10,18.0/10,18.1/10
- Flow rate : 1.0 ml /minutes
- Column oven temperature : Ambient
- Detector : UV-254 220 and 366 nm
- Standards stock concentratio : 10 mg/ml Working –spiked volume 10 µl
- Total runtime : 20.1 minutes

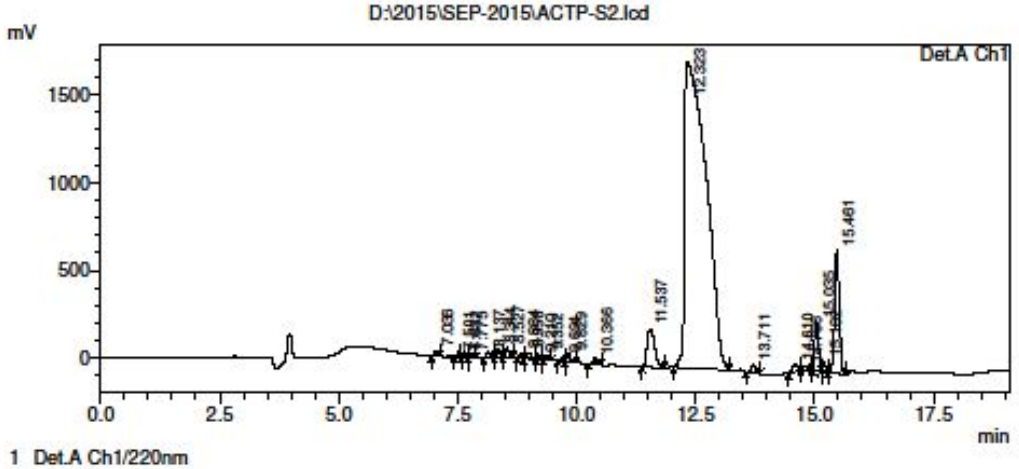
==== Shimadzu LCsolution Analysis Report ====

D:\2015\SEP-2015\ACTP-S2.lcd

Acquired by	: Admin
Sample Name	: 18A-ACTP-S1
Sample ID	: 18A-ACTP-S1
Tray#	: 1
Vail #	: 16
Injection Volume	: 20 uL
Data File Name	: ACTP-S2.lcd
Method File Name	: Bharath university.lcm
Batch File Name	:
Report File Name	: Default.lcr
Data Acquired	: 9/23/2015 11:58:58 AM
Data Processed	: 9/23/2015 12:23:24 PM

<Chromatogram>

D:\2015\SEP-2015\ACTP-S2.lcd



1 Det.A Ch1/220nm

PeakTable

Detector A Ch1 220nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	7.036	117447	32370	0.175	0.906
2	7.501	60306	13213	0.090	0.370
3	7.632	87499	21562	0.130	0.604
4	7.775	94682	22486	0.141	0.629
5	8.137	177340	32932	0.264	0.922
6	8.344	220294	49096	0.328	1.374
7	8.527	267413	53830	0.398	1.507
8	8.834	170504	31392	0.254	0.879
9	8.958	255463	31697	0.380	0.887
10	9.210	118703	22326	0.176	0.625
11	9.352	151545	27855	0.225	0.780
12	9.694	132331	24884	0.197	0.697
13	9.829	269510	49582	0.401	1.388
14	10.366	182205	38192	0.271	1.069
15	11.537	2410037	216625	3.583	6.064
16	12.323	55012461	1745573	81.792	48.861
17	13.711	301387	47556	0.448	1.331
18	14.610	329849	44205	0.490	1.237
19	14.766	163373	21357	0.243	0.598
20	15.035	1609759	278949	2.393	7.808
21	15.182	268566	58721	0.399	1.644
22	15.461	4858015	708122	7.223	19.821
Total		67258687	3572524	100.000	100.000

Valued for their aromatic, savory, or medicinal characteristics, herbs come from plants or various parts of plants and possess certain chemical substances that have effects on the body. Herbal medicine continues to influence the medicines of today and up to 25% of all prescription drugs in the United States have at least one active ingredient that comes from plant extracts or synthesized plant compounds[6]. According to the WHO as many as 4 billion people or 80% of the earth's population are estimated to use some form of herbal medicine in their health care. Moreover, the key bioactive compounds and the role of medicinal plants in Ayurvedic systems of medicine in India has been an increase in demand for the phytopharmaceutical products of Ayurveda in western countries, because of the fact that the allopathic drugs have more side effects. Different types of plant parts are used for the Ayurvedic formulation; overall out line of those herbal scenario and its future prospects for the scientific evaluation of medicinal plants is being used by traditional healers. As much as possible importance is also given for the taxonomic literature.[7.]

The HPLC analysis was performed using a HPLC-LC-2010 SHIMADZU with LC-UV-100 UV detector, C18-250×4.6 -5µ 100 A^0 was used for the chromatographic separations. The mobile phase consisted of solvent mixtures [Methanol: Water (95:5)] ratio with a flow rate of 1.0ml/min and ambient column temperature with 10 mg /ml sample concentration. The injection volume was 20 µl, and UV detection was effected at 366 nm Stock solutions of the isolated compounds were prepared in HPLC grade methanol at a concentration of 100 μgmL^{-1} and stored in a refrigerator until use. All samples were stored at 4°C and were filtered through a 0.45 µm filter before undertaking HPLC analysis. The preliminary screening test may be useful in the detection of bioactive principles and subsequently may lead to the drug discovery and development. HPLC analysis of chloroformic extract of ACTP was done. The HPLC chromatograph will help as standard chromatogram in future studies, comparing the retention time of isolated compounds with given literatures[4]. HPLC analysis of Chloroformic extract of ACTP was carried out with the mobile phase methanol : water in the ratio 95:5 gave a total of 22 peaks at retention time 7.036, 7.501, 7.602,7.775, 8.137,8.344, 8.527, 8.834, 8.958, 9.210, 9.352, 9.694, 9.829, 10.366, 11.537, 12.323, 13.711, 14.610, 14.766, 15.035, 15.182, 15.461 (Figure). The highest peak was seen at the retention time 12.323 minute Height 1, 74,557, Area percentage 81.792 and Height percentage 48.86.[5.]

4. References

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