

Chromatographic profiling using RP-HPLC and Estimation of Biochemical Parameters of *Saussera lappa*

Thara K. M* and K. F. Zuhra

Department of Life Sciences, University of Calicut, Kerala, India

ABSTRACT

The current phytochemical investigation deals with the chromatographic fingerprinting of the methanolic extract using High Pressure Liquid Chromatography using a RP C-13 column and a Photo Diode Array Detector of the dried roots of *S.lappa* for the detection of the chemical constituents in the plants. Efforts were also made for the quantitative determination of the biochemical parameters for studying the biological efficacy of the plant. The current study revealed that the plant is very much rich in Phenolics and the results also denote that the plant possess a significant F/P ratio which is very near to the standard value one which implies the biological potency of the species. The HPLC profile enabled the separation of 19 constituents from the methanolic extract.

Keywords: *Saussera lappa*, HPLC, F/P Ratio, Biochemical parameters.

INTRODUCTION

Traditional medicines have been used for many centuries by a substantial proportion of the population of India[1].The World Health Organization (WHO) estimated that 80% of the populations of developing countries rely on traditional medicines, mostly plant drugs, for their primary health care needs[2],[3]. *Saussera lappa* belongs to the family Asteraceae is an important endangered medicinal plants. On the other hand, the dried root of the plant is a popular medicinal material for treating various gastrointestinal diseases[4]



Dried Roots of *S.lappa*

MATERIALS AND METHODS

Plant Collection

The dried roots of *Saussera lappa* were collected from local market, Calicut in Dec 2010 and authenticated from the Botany Department of Calicut University, Kerala. Voucher specimen is preserved in the herbarium of College. The plant material was then shade dried and powdered.

Preliminary Phytochemical screening

Methanolic extract in the concentration 100 mg ml^{-1} was used for the detection of major class of chemicals like carbohydrates, phenols, flavonoids, tannins, alkaloids, glycosides, saponins, anthraquinones and amino acids present in the flowers by performing specific chemical tests[5].

Biochemical Parameters**Estimation of Phenolics**

The estimation of the phenolic compound in the plant was performed using modified Bray and Thorpe Method[6]. The amount was calculated in milligram equivalence of Gallic acid.

Estimation of Flavonoids

The amount of total flavonoidal compounds in the plant was calculated using Aluminium Chloride method [7]. The results were expressed in milligram equivalence of Quercetin

Calculation of F/P Ratio

F/P ratio of the dried roots were calculated from the two above said values

High Pressure Liquid Chromatography (HPLC)**Instrumentation**

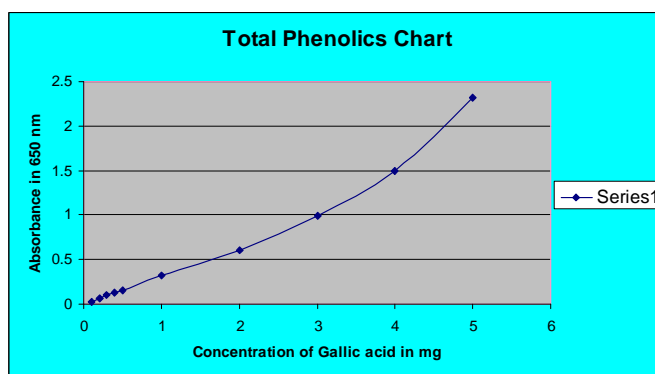
The high-pressure liquid chromatographic system consists of Shimadzu LC – 10ATVP pump, a valve type injector, Shimadzu SPD10 AVP model Photo Diode detector (Shimadzu, Tokyo, Japan), Phenomenex Luna C 18 (250×4.6 nm) column with a particle size of 5μ . Acetonitrile, methanol (HPLC grade, E.Merck, India) in the ratio 5:5 were used for the analysis

RESULTS**Preliminary Screening**

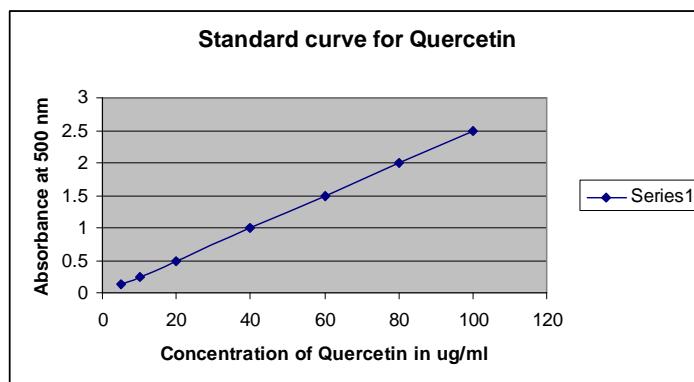
No	Checked for	Name of the test	Quantity Present
1	Phenolics	Lead acetate Test	+++
2	Flavonoids	Shinoda's Test	++
3	Alkaloids	Dragendroff's Test	--
4	Saponins	Foam Test	+
5	Glycosides	Molisch's Test	--
6	Proteins	Biuret Test	--
7	Terpenoids	Tin and Thionyl Chloride Test	--

Estimation of total Phenolics

The total phenol present in the sample were seems to be 25.5 % mgEq of Gallic acid

**Estimation of total Flavonoids**

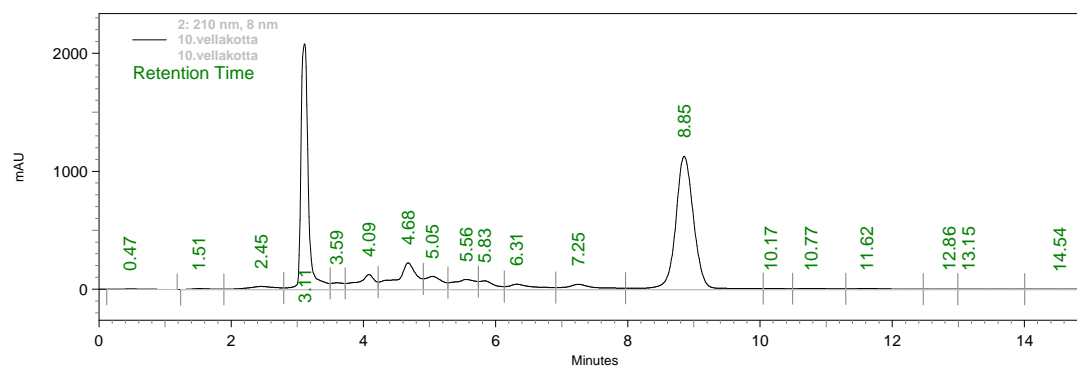
The total phenol present in the sample were seems to be 8.5 % mgEq of Quercetin



Calculation of the F/P ratio

The F/P ratio was found to be 0.33.

HPLC CHROMATOGRAM of the Methanolic extract of the dried roots of *S.lappa*



HPLC Fingerprints under 210 nm

Pk #	Retention Time	Area	Area %	Height	Height %	Start Time	Stop Time
1	0.47	83455	0.15	2715	0.07	0.12	1.18
2	1.51	162672	0.29	7820	0.19	1.24	1.89
3	2.45	772062	1.39	26129	0.65	1.89	2.79
4	3.11	16248598	29.27	2080758	51.42	2.79	3.50
5	3.59	730662	1.32	56454	1.40	3.50	3.72
6	4.09	2329265	4.20	127042	3.14	3.72	4.22
7	4.68	4757625	8.57	225691	5.58	4.22	4.91
8	5.05	1996351	3.60	110719	2.74	4.91	5.28
9	5.56	1962200	3.53	84823	2.10	5.28	5.74
10	5.83	1181682	2.13	73405	1.81	5.74	6.13
11	6.31	1245583	2.24	45161	1.12	6.13	6.91
12	7.25	1338165	2.41	44199	1.09	6.91	7.97
13	8.85	21561411	38.84	1129545	27.91	7.97	10.05
14	10.17	182892	0.33	7350	0.18	10.05	10.50
15	10.77	295120	0.53	7157	0.18	10.50	11.30
16	11.62	327245	0.59	8078	0.20	11.30	12.47
17	12.86	84673	0.15	2929	0.07	12.47	12.99
18	13.15	122024	0.22	2926	0.07	12.99	14.01
19	14.54	71216	0.13	1572	0.04	14.01	14.83
Totals		55508747	100.00	4046708	100.00		

The HPLC chromatogram revealed the presence of 19 compounds in the extract under 210 nm. Among the constituents the compounds at Rt 3.11 and 8.85 were the major which comprises around 29.2% and 38.8 % among the total constituents at this detection nanometer.

CONCLUSION

From the present study we conclude that the medicinal plant *Saussurea lappa* contains a larger amount of phenolics and Flavonoids which support the biological potency of the plant. The HPLC profile also shows the presence of various chemical compounds in the plant. The F/P ratio was higher than 0.30 which shows that the plant material is moderately capable of converting the phenolics to Flavonoids. The HPLC chromatogram we have developed can be used for the routine quality analysis of the plant and also for the identification of the genuine plant from its adulterants.

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