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STUDY OF PIPPALI RASAYANA: AN ANALYTICAL APPROACH

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ABSTRACT

Due to multipurpose use and effect of *Rasayana* therapy it is considered as most prospective area for research. *Rasayana* in ancient India was much developed than today. '*Rasayana-tantra*' an integral part of *Astanga Ayurveda* has been well discussed in classical text of *Ayurveda*. In present scenario these concepts attracts the scientific communities for research because of their preventive and curative action. The *Rasayana* properties of *Pippali-Rasayana* were tested by modern techniques. For this the antioxidants of *Pippali-Rasayana* was examined by quantitative and qualitative analysis of phenolic extracts of *Pippali-Rasayana* by photometry and High-performance liquid chromatography (HPLC) methods respectively. The presence of antioxidant compounds in *Pippali Rasayana* indicates its *Rasayana* properties.

Keywords: Photometry, HPLC, *Rasayana*, Antioxidants, *Pippali-Rasayana*

INTRODUCTION

Ayurveda is our old age science of life and medicine is on the go of popularizing and has seen a tremendous increase in the attention level and curiosity of people globally since past 3 decades. The desire to live, one of the basic instincts has been common to all living creatures ever since the first unicellular organism evolved on this graceful earth. This desire itself was the cause élan vital which lead to the successful evolutionary progress into more structurally and functionally developed unicellular and later into multi cellular organism. Man, the most developed and sophisticated living being on earth, so considered due to his tremendous intellectual abilities is in no way at par from this. On the contrary, he is a step ahead, in the sense, desires not only to live, but to live a long, happy and disease free life as far as possible. The times have changed since revered sages had described about the means and methods of leading such a life, emphasis being laid on *Swasthavrita*, *Sadvrita* and mainly the avoidance of

Prajnaparadha, the root cause of all diseases[1]. In the blown up human civilization of present era, in the midst of increased pronicity and various stresses, it is natural to anticipate majority of population as having undesired health problems resulting in short life span. This was envisaged by our ancient revered *Acharyas* and they have found the solution thousands of years ago, after an extensive search to explore the means and methods for *Hitayu*, *Sukhayu* and *Dirghayu* in the concept of *Rasayana* which not only helped attain longer, healthier life but also helped curtail some of the dreadful diseases[2].

It has been established that oxidative stress is among the major causative factors in induction of many chronic and degenerative diseases including atherosclerosis, ischemic heart disease, ageing, diabetes mellitus, cancer, immunosuppressant, neurodegenerative diseases and others[3]. A great number of aromatic, medicinal, spice and other plants contain chemical compounds exhibiting antioxidant properties. Oxidative process is one of the most important routes for producing free radicals in foods, drugs and even in living systems[4,5]. The most effective path to eliminate and diminish the action of free radicals which cause the oxidative stress is anti-oxidative defense mechanisms. Antioxidants are those substances which possess free radical chain reaction breaking properties. Recently there has been an upsurge of interest in the therapeutic potential medicinal plants as antioxidants in research[6]. Antioxidant reduces oxidative stress-induced tissue injury. Among the numerous naturally occurring antioxidants; ascorbic acid, carotenoids and phenolic compounds are more effective[7]. They are known to inhibit lipid peroxidation (by inactivating lipoxygenase), to scavenge free radicals and active oxygen species by propagating a reaction cycle and to chelate heavy metal ions[8]. The study done on medicinal plants and vegetables strongly supports the idea that plant constituents with antioxidant activity are capable of exerting protective effects against oxidative stress in biological systems[9,10].

On continuation of our experimental work for the search of antioxidant activity of medicinal plants, we studied extracts of *Pippali-Rasayana*[11]. The presence of phenolic compound was evaluated during the course of work. The qualitative and quantitative estimation of phenolic compounds in *Pippali-Rasayana* has been done by HPLC and photometry methods. The assessment of such properties remains an interesting and useful task, particularly for finding new sources for natural antioxidants[7]. With the advancement of technology came in modernized machine and manufacturing technique which result in increased efficacy & palatability of ancient formulations ultimately giving wonderful result to the patients, hence giving new horizons to the *Ayurvedic* industry.

Material and methods:

Fruits of *Piper longum* L. of large variety were selected and collected from local market in Varanasi. It was authenticated by consulting the senior teachers of Department of Dravyaguna, Faculty of Ayurveda, Institute of Medical Sciences, Banaras Hindu University, Varanasi, and also by consulting the authentic literature available on *P. longum*. The selected *P. longum* fruits were washed with distilled water and dried in indirect sunlight. After that these were powdered and stored in an airtight container for further use.

A healthy *Butea monospermum* of red variety is selected and authenticated by consulting the senior teachers of Department of Dravyaguna, faculty of Ayueveda, Institute of Medical Sciences, Banaras Hindu University, Varanasi. Then the parts (leafs, flowers, stem, and root bark) of *B. monospermum* were collected from the forest of Vindhyanchal mountain region of Mirzapur district, Uttar Pradesh, India. The collected materials were washed with distilled water and dried in indirect sunlight. By these collected parts of *B. monospermum*, alkali water is prepared by following the guidelines of Susruta-samhita[12].

Pippali-Rasayana was prepared by impregnation of *Pippali* powder with alkali water (*kshara* water) of *Palash*[13]. The number of impregnation is not mention by Acharya Caraka[13]. But Acharya Yogendra Nath Sen has mentioned that the number of

bhawana should be seven6. The *Pippali* powder is impregnated with alkali water of *B. monospermum* for seven times and then it was fried with cow ghee. For evaluation of qualitative and quantitative antioxidant content of *P. longum* and *Pippali-Rasayana* four samples were separated these are -

1 – Powdered *P. longum*.

2 – *P. longum* after one impregnation with alkali water.

3 – *P. longum* after seven impregnations with alkali water.

4 – Seven time impregnated *P. longum* after fried with cow ghee.

Qualitative analysis i.e. total phenol content through spectrophotometry:

Material used: Test tubes, Folin reagent, sodium bicarbonate, spectrophotometer etc.

Methods: Samples were kept to 50% methanol water for over night.100µl of each sample was kept in separate test tube and maintained the volume up to 1ml, 0.5ml of folin reagent was added there after fallowed by addition if 1ml of 20% Na_2CO_3. Blue color was appeared. The whole setup was kept for 20 minute at room temperature. 10ml DW was added and then OD was taken at 725nm in spectrophotometer. Standard curves of gallic acid were prepared and total phenolic concentrations were calculated in terms of gallic acid equivalents. Statistical analysis was performed by computer software SPSS16. The data were subjected to ANOVA.

Observation and Result

Total phenol content of samples

Calculation

OD of each of four samples was taken for three times. Table1.

The mean of OD of each sample was calculated by applying the ONE WAY ANOVA.

Total phenol content = 12.822 x OD mg phenolic per gram of fresh samples equivalent to gallic acid.

The phenol content of sample 1 was found to be 1.7267 mg phenolic per gm of fresh samples equivalent to gallic acid.

Whereas phenol content of sample 2 was 1.5500 mg, sample 3 was 1.4400 mg and sample 4 was 2.2600 mg phenolic per gm of fresh samples equivalent to gallic acid.

Result

The phenol content of sample 1 was found to be 1.7267 mg phenolic per gm of fresh samples equivalent to gallic acid. Whereas phenol content of sample 2 was 1.5500 mg, sample 3 was 1.4400 mg and sample 4 was 2.2600 mg phenolic per gm of fresh samples equivalent to gallic acid.

Out of all the four samples, the sample 4 showed highest amount of phenolic acid compared to the other samples. The second best sample was sample 1fallowed by sample 2 and sample 3. (Table 1 & Fig. 1)

Qualitative analysis of phenol compounds through HPLC

Material used: Ethanol water, screw-capped tubes, whatman filter paper, Winchrom integrator, HPLC. system (Shiadzu Corporation, Kyoto, Japan).

Methods: Two grams of each sample were mixed in 5 ml of ethanol water (80 : 20 V/V). The prepared samples were placed in separate screw-capped tubes and the suspensions were subjected to ultrasonication (Branson Sonifier, USA) at 60% duty cycles for 15min at 4 °C followed by centrifugation at 7500 rev/min for 15min. The supernatant was subjected to charcoal treatment to remove pigments in each of the samples and transferred to screw-capped glass tubes after passage through Whatman filter paper no.1. The residue was re-extracted twice and the supernatant was pooled prior to evaporation under vacuum (Buchi Rotavapor Re Type). The dried samples were resuspended in 1.0ml high performance liquid chromatography (HPLC) grade methanol by vortexing and stored at 4°C for analyses. All of the samples were prepared in triplicate.

HPLC analysis

HPLC of the samples was performed according to Singh et al. (2002) with an HPLC System (Shiadzu Corporation, Kyoto, Japan) equipped with two Shi-

madzu LC 10 ATVP reciprocating pumps, a variable UV–VIS detector (Shimadzu SPD-10AVP) and a Winchrom integrator (Winchrom). Reverse phase chromatographic analyses were carried out under isocratic conditions using a C-18 reverse phase HPL Column (250 x 4:6mm id, particle size 5µm Luna 5µ C-18 (2), Phenomenex, USA) at 25 °C. Running conditions comprised injection of a volume of 5 µl, mobile phase methanol: 0.4% acetic acid (80:20,v/v), flow rate 1ml/min, detection at 290nm. Samples were filtered through membrane filter (poresize 0.45µm, Merck) prior to injection into the sample loops. At least three runs were performed for each sample. Tannic, gallic, chlorogenic, ferulic, cinnamic and salicylic acids were used as internal and external standards. Phenolic compounds and salicylic acid present in the samples were identified by comparing retention time (RT) of standards and by co-injection. Concentrations were calculated by comparing peak area as of the reference compounds with those in the samples run under the same elution conditions. Statistical analysis was performed by computer software SPSS 16. The data were subjected to ANOVA. (Table 1, fig. 1)

Results:

The HPLC analysis of samples yielded many peaks out of which only few are identified.

Sample 1

In HPLC analysis of sample No. 1 yielded total 22 peaks out which only 4 were identified i.e. Shikimic acid (Rt 2.623min), gallic acid (Rt 3.798), rutin (Rt 9.507) and salicylic acid (Rt 18.98). (Fig. 2)

Sample 2

In HPLC analysis of sample No. 2 which contained Pippali after one impregnation with *Palash* alkali water yielded total 5 peaks out of which only one was identified i.e. Rutin (Rt 9.507). (Fig. 3)

Sample 3

In HPLC analysis of sample No. 3 which contained *Pippali* after seven impregnation with *Palash* alkali, water yielded total 18 peaks out of which only four were identified i.e. Gallic acid (Rt 3.798), trans-chlorogenic acid (Rt 4.989), syringic acid (Rt 7.803) and myrecetin (Rt 16.57). (Fig. 4)

Sample 4

In HPLC analysis of sample No. 4 which contained *Pippali* after seven impregnation with *Palash* alkali water and fried in cow ghee yielded total 16 peaks out of which only nine were identified i.e. Shikimic acid (Rt 2.623), gallic acid (Rt 3.7980), trans chlorogenic acid (Rt 4.989), syringic acid (Rt 7.803), Rutin (Rt 9.507), p – coumaric acid (Rt 10.75), synapic acid (Rt 11.967), myrecetin (Rt 16.57) and salicylic acid (Rt 18.98). (Fig. 5)

DISCUSSION

Ayurveda system of medicine is probably the first which effort to protect life from disease and aging in the form of *Rasayana*. *Rasayana* definitely have power to prevent or delay the aging process and age related disorders. How these *Rasayana* prevent aging and age related disorder is well mentioned in ayurvedic literature in the context of *Rasayana-tantra*[14,15]. But it is the necessity of time that it should be proved on modern scientific parameters. Results indicate that the present *Rasayana* preparation contains considerable amounts of phenolic compounds. Phenolic compounds are well knoem for their antioxidants properties i.e. protect body from the free radical damage which contributes in aging and several degenerative processes.

In the modern science among the theories of aging the theory of free radical damage is most popular and most acceptable[16]. In free radical theories how free radical form in body, it is common knowledge by now a days that oxygen, though crucial for life process produces highly reactive substance called free radical, as by product of ATP. These free radical form in body by the consumption of unwholesome diet or free radical diet. So due to unwholesome diet the production of free radical in body get increases and these free radicals can damage any part of body and may result in many serious diseases. Free radicals are produced in mitochondria, and damage protein, lipids, nucleic acids of cells and mitochondria themselves – especially their DNA. The resultant DNA alterations and mutations lead to ageing and age-related degenerative diseases like

progressive dementia, vision loss, neurosensory deafness and abnormal cardiac and renal functions[17]. They also act upon RNA, causing accumulation of post-mitotic errors, and defective protein synthesis. Though Tissue damage caused by oxygen radical is often called oxidative damage, and factors that protect against oxygen radical damage are known as antioxidant. These by reducing the rate of chain initiation, by braking the chain propagation of radical damage and by free radical scavenging activities i.e. washout the free radical from the body protect against oxygen radical damage.

While regarding *Pippali* the most authentic book of Ayurvedic literature '*Caraka-Samhita'* mentioned that excess use of *Pippali* for longer duration is injudicious[18] because of qualities like immediately exhibit their bad or good effects, if constantly used leads to consequent accumulation of dosas. Yogendranath Sen clarified that, single use of *Pippali* is not injudicious but its use with other drug is injudicious. Further, *Acharya-Caraka* in context of *Rasayana,* mentioned prolong and excess use of *Pippali* in form of *Pippali-Rasayana*, *Vardhamana-Pippali Rasayana* etc. as *Pippali Rasayana* prepares by impregnation of *Pippali* by *Palash kshara*[11].

CONCLUSION

This may be concluded that *Pippali-Rasayana* as prepared according to *Caraka-Samhita*, contains phenolic compounds and phenolic compounds definitely posses' antioxidants properties. In scientific researches' it is found that *Rasayana* are the rich source of antioxidants and *Rasayana* exert their effects through antioxidant compound and mechanism. Hence concepts, principles and methods described in *Samhitas* have sound footing, reasoning and logic as proven by present experimental evidences.

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Table 1: Photometric evaluation of *Pippali* and *Pippali Rasayan*

Samples	Readings	Percentage transmission	Percentage absorption/ OD
0 (blank)	1st Reading	99.9	0.00
Sample 1	1st Reading	73.8	0.132
	2nd Reading	72.2	0.136
	3rd Reading	73.0	0.137
Sample 2	1st Reading	76.7	0.115
	2nd Reading	74.8	0.126
	3rd Reading	75.3	0.123
Sample 3	1st Reading	76.9	0.114
	2nd Reading	75.0	0.125
	3rd Reading	74.8	0.126
Sample 4	1st Reading	62.6	0.203
	2nd Reading	70.7	0.150
	3rd Reading	65.7	0.183

Fig. 1

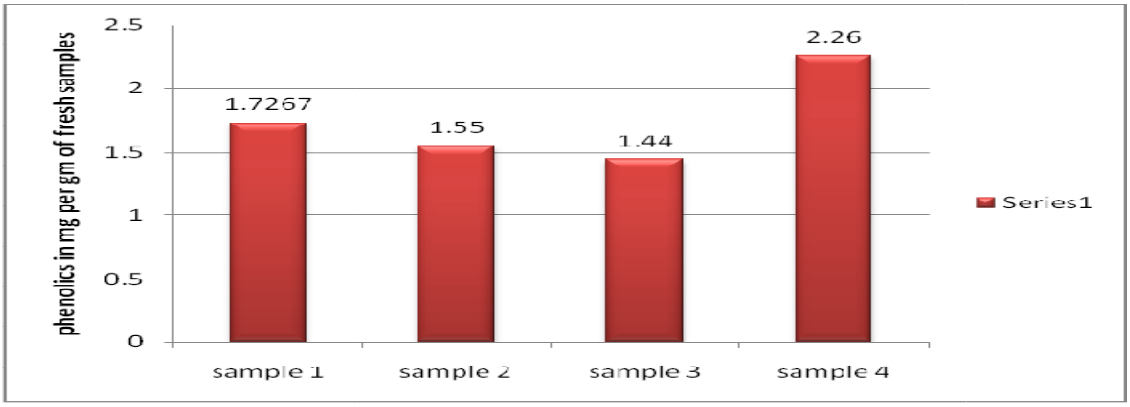
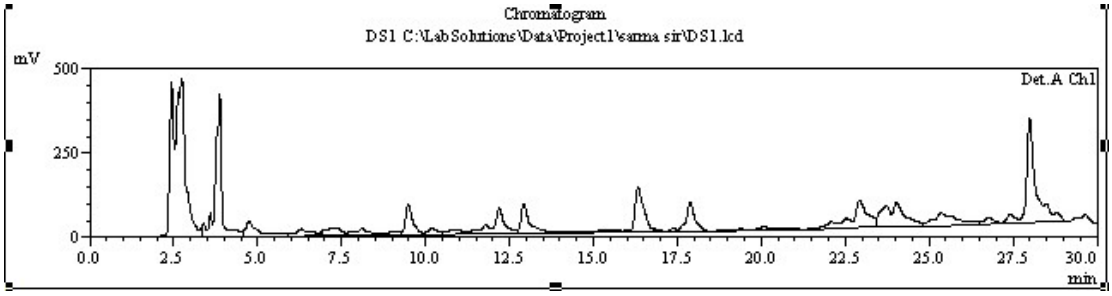


Fig. 2: HPLC analysis of sample 1

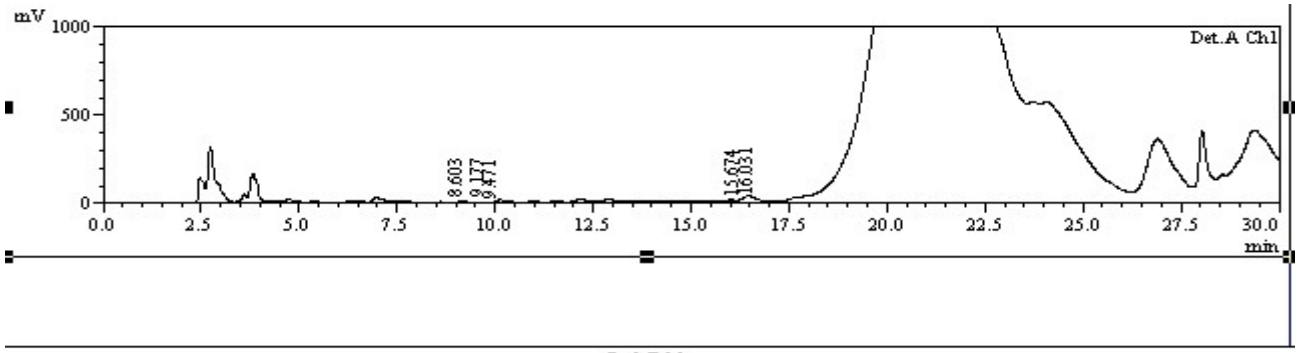


Peak Table

Detector A Ch1 254nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	2.466	12859246	394643	26.613	21.645
2	3.849	5784139	367898	11.971	20.178
3	4.742	1270871	40976	2.630	2.247
4	6.300	721514	19944	1.493	1.094
5	7.268	785940	23906	1.627	1.311
6	8.113	586171	18801	1.213	1.031
7	9.486	1664706	81831	3.445	4.488
8	10.200	355058	18154	0.735	0.996
9	10.947	440845	11895	0.912	0.652
10	12.199	2031554	71714	4.204	3.933
11	12.935	1650249	79383	3.415	4.354
12	15.639	544939	8949	1.128	0.491
13	16.335	2510103	117395	5.195	6.439
14	17.884	1671055	84230	3.458	4.620
15	18.824	145917	3528	0.302	0.193
16	20.075	223283	9765	0.462	0.536
17	20.453	250746	6595	0.519	0.362
18	22.937	2963935	77706	6.134	4.262
19	23.708	3158849	63196	6.538	3.466
20	25.361	1774414	36274	3.672	1.989
21	26.788	405166	18338	0.839	1.006
22	27.994	6520056	268142	13.494	14.707
Total		48318754	1823263	100.000	100.000

Fig. 3 - PLC analysis of sample 2

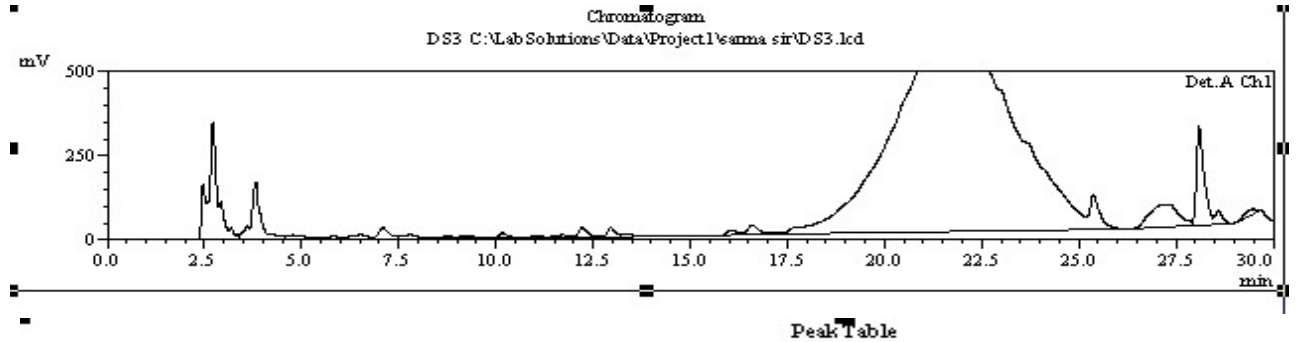


Peak Table

Detector A Ch1 254nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	8.603	62663	3977	27.711	26.059
2	9.177	86696	4171	38.339	27.329
3	9.471	14945	1370	6.609	8.977
4	15.674	15277	1233	6.756	8.076
5	16.031	46550	4511	20.585	29.560
Total		226132	15262	100.000	100.000

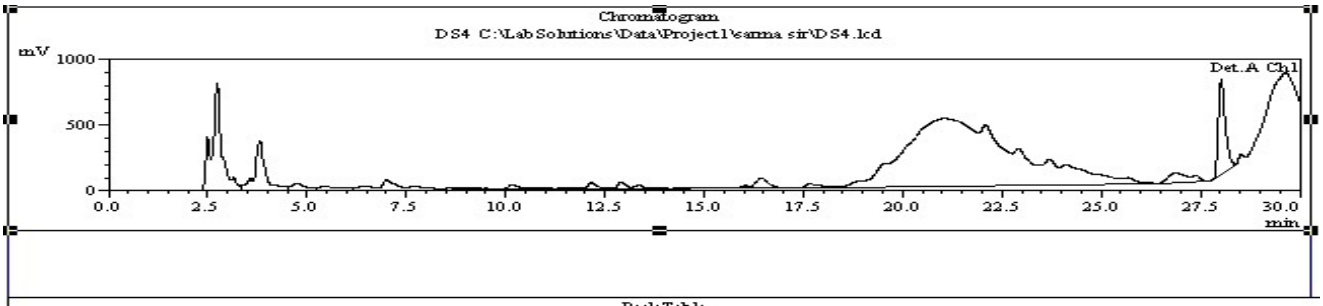
Fig. 4 - PLC analysis of sample 3



Peak Table

Detector A Ch1 254nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	2.484	6470553	143325	4.140	9.768
2	3.808	3016553	157416	1.930	10.728
3	4.833	420998	12878	0.269	0.878
4	5.823	313679	7445	0.201	0.507
5	7.091	1006355	29475	0.644	2.009
6	7.790	353195	11480	0.226	0.782
7	8.728	120571	5573	0.077	0.380
8	9.283	205712	6875	0.132	0.469
9	10.159	232616	12301	0.149	0.838
10	11.073	109365	3423	0.070	0.233
11	12.223	630136	26584	0.403	1.812
12	12.962	578011	26051	0.370	1.775
13	14.687	63358	3518	0.041	0.240
14	16.602	982540	28680	0.629	1.955
15	21.833	133244298	644090	85.260	43.896
16	27.231	3600091	69053	2.304	4.706
17	28.115	4575200	267140	2.928	18.206
18	29.496	356093	11991	0.228	0.817
Total		156279324	1467300	100.000	100.000



Peak Table

Detector A Ch1 254nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	2.725	14649751	707934	9.823	26.253
2	3.802	7382244	362404	4.950	13.439
3	4.751	1475644	49180	0.989	1.824
4	5.455	1296986	27402	0.870	1.016
5	7.020	3605805	70703	2.418	2.622
6	8.638	351085	15902	0.235	0.590
7	9.174	545729	14339	0.366	0.532
8	10.181	838922	34578	0.562	1.282
9	10.953	268885	12271	0.180	0.455
10	12.174	2370736	41478	1.590	1.538
11	14.584	237554	8852	0.159	0.328
12	16.447	2076934	74452	1.393	2.761
13	17.671	720923	27481	0.483	1.019
14	21.040	101276448	514196	67.905	19.068
15	26.891	2950187	71327	1.978	2.645
16	28.034	9096070	664104	6.099	24.627
Total		149143903	2696604	100.000	100.000

Fig. 5 - PLC analysis of sample 4

Source of Support: Nil
Conflict Of Interest: None Declared

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