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ISSN: 2322 - 0902 (P)
ISSN: 2322 - 0910 (O)

International Journal of Ayurveda and Pharma Research

Research Article

STANDARDISATION OF POLYHERBAL DRUGS-CERVILON AND LUMBATON CAPSULES

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ABSTRACT

Standardization of Ayurvedic formulations is an important step for the establishment of a consistent biological activity, a consistent chemical profile, or simply a quality assurance program for production and manufacturing of herbal drugs. This article reports on polyherbal drugs Cervilon and Lumbaton soft gel capsule is an effective herbal Indian Ayurvedic medicine used for the neck pain and skeleto-muscular problems such as cervical spondylosis, cervical injuries, lumbar spondylosis, lower back pain, sciatica, numbness. Samples were collected from AVN Ayurveda formulations (P) Ltd. Madurai. The drugs has been standardised on the basis of physio-chemical parameters, antifungal and antimicrobial activity. High performance thin layer chromatography (HPTLC) fingerprint profiles were carried out to analysis the identity, purity and strength of the drugs. The chemical parameter analyses such as Acid value, Saponification value, Peroxide value, Iodine value are calculated and it was observed that all the values within the limits. Preliminary phytochemical analysis of the methanol extract of soft gel capsules of Cervilon and Lumbaton revealed the presence of various bioactive components which include carbohydrate, protein, alkaloids, flavanoids, and saponins. There is no fungal count was observed in plates. Antimicrobial activity was observed in soft gel capsules. They showed significant inhibition in disease causing bacteria. Selected poly herbal capsule have passed through all the WHO parameters which were tested. So it can be concluded that use of capsule was safer and ready to use.

KEYWORDS: Standardization, Cervilon, Lumbaton, spondylosis, HPTLC

INTRODUCTION

Infectious disease is continued to be a serious burden around the world, in developing and industrialized countries. Over usage of allopathic medicines produce undesired side effects. Hence, the traditional medicinal practitioners and scientists are turning towards medicinal plant and traditional system of medicines such as Siddha, Ayurveda, Unani, homeopathy medicines and formulations to reduce the side effect and toxicity. The world Health Organisation (WHO) supports the use of traditional medicine provided they are proven to be efficacious and safe. The use of herbal medicine for treatment of disease and infections is as old as mankind (WHO, 1985). Herbal medicines are becoming popular because of multidrug resistance from indiscriminate usage of antibiotics. Natural products isolated from higher plants have been providing clinically active drugs which made the scientists to identify the potent and effective antimicrobial agents of plant origin to replace the antibiotics. [1]

Antibiotics provide the main basis for the therapy of microbial (bacterial and fungal) infections. Since the discovery of these antibiotics and their uses as chemotherapeutic agents there was a belief in the medical fraternity that this would lead to the eventual eradication of infectious diseases. However, overuse of antibiotics has become the major factor for the emergence and dissemination of multi-drug resistant strains of several groups of microorganisms[2]. In recent years, plant derived products are increasingly being sought out as medicinal products, nutraceuticals and cosmetics and are available in health food shops and pharmacies over the counter as self-medication or also as drugs prescribed in the non-allopathic systems[3,4]. Herbal medicines widely used in health-care in both developed and developing countries are complex chemical mixtures prepared from plants and are limited in their effectiveness because they are poorly absorbed when taken orally [5]. Herbal formulations have reached widespread acceptability as therapeutic agents for diabetics, arthritics, liver diseases, cough remedies, memory enhancers and adoptogens[6]. As per WHO definition, there are three kinds of herbal medicines: raw plant material, processed plant material and medicinal herbal products. Herbal drugs are finished labelled products that contain active ingredients such as aerial or underground parts of plant or other plant material or combination thereof, whether in the crude state or as plant preparations. The use of herbal medicines has increased remarkably in line with the global trend of people returning to natural therapies[7]. Herbal medicine products are dietary supplements that people take to improve their health and are sold as tablets, capsules, powders, teas, extracts and fresh or dried plants[8]. Herbals are traditionally considered harmless and increasingly being consumed by people without prescription. However, some can cause health problems, some are not effective and some may interact with other drugs. Standardization of herbal formulations is essential in order to assess the

quality of drugs, based on the concentration of their active principles physical, chemical, phyto-chemical, standardization, and In-vitro, In-vivo parameters[9]. Standardization of Ayurvedic formulations is an important step for the establishment of a consistent biological activity, a consistent chemical profile, or simply a quality assurance program for production and manufacturing of herbal drugs.

MATERIALS AND METHODS

The sample Cervilon (I) and Lumbaton (II) was collected from AVN Ayurveda formulations (P) Ltd. Madurai, Tamilnadu, India. Sample was squeezed and then added ethanol. It was mixed by using cyclometer and then filtered for carried out the following test.

Evaluation of parameters for Ayurvedic capsule sample I & II

Iodine value, Peroxide Value, Saponification Value, Acid Value were estimated in sample I and II. Preliminary phytochemical analysis of the ethanol extract of soft gel capsules of Cervilon and Lumbaton. Total bacterial and fungal count were also estimated in both sample.

Antimicrobial activity

Target organism [*Salmonella typhi, Escherichia coli, Pseudomonos aerogenus, Vibrio cholera, Staphylococcus aureus, Bacillus subtillus*] used in the study were obtained from AVN Ayurveda formulation microbiology laboratory. They are stored at 4˚c and subsequently sub- cultured once in a month using nutrient agar medium. They are maintained as nutrient broth culture for the study. Wells were cut in nutrient agar plate.24hours culture of were seeded in the nutrient agar plate**.** 20µl of concentration of the methanol extract were loaded in the well. The ethanol extract was treated with dimethyl sulfoxide (DMSO), 1ml to add and suppress the activity of methanol. The plates were incubated at 37˚c for 24hours. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the well.

HPTLC Procedure

Silica gel 60F 254 HPTLC plates in the format of 4×10cm are used. Develop plate with 20ml of methanol per trough in a 4×10cm.Twin trough chamber to the upper edge equilibrate plate with lab atmosphere in a suitable container providing protection from dust and fumes. Plates are handled either on both sides or on the top edges.

Samples are applied as bands using a suitable instrument. Developing solvents consisting of more than one component are prepared by measuring the required volume of each component separately and transferring them into a solvent bottle of appropriate size. The appropriate volume of developing solvent (4×10cm) was prepared. The chamber was opened and placed the correctly sized piece filter paper in the rear trough. The solvent was poured into chamber so that filter paper was wetted and adheres to rear wall of TLC. The chamber was tilted to the side so that the solvent volume in both troughs equalizes. The chamber was equilibrated for 2 hours. The plate was inserted into the front trough. The layer faces the filter paper and the back of the plate rests against front wall of TLC. The lid was replaced and develops plate to the mark. The plate was removed and dried it for 5minutes in a steam of cold air..After each development remaining mobile phase and filter paper are discarded..Prior to preparation for the next run the chamber is dried and cleaned. Transfer of reagents for derivation of samples on a HPTLC plate may be accomplished by spraying or dipping. Spraying is done in a TLC spray cabinet or in the fame hood. Change the bottle of the sprayer with up to 5ml of reagent. Place plate in a spray cabinet and spray plate until it is homogenously covered with the reagent. Dry plate with cold air and proceed with handling. Each developed plate is documented under 254nm.

RESULTS AND DISCUSSION

As part of standardisation procedure, the cervilon and lumbaton soft gel capsules were tested for chemical analysis such as Acid value, Saponification value, Peroxide value, Iodine value and also subjected microbiological analysis. Chemical parameters are calculated and mentioned in table 1, it was observed that all the values within the limits. Preliminary phytochemical analysis of the ethanol extract of soft gel capsules of Cervilon and Lumbaton revealed the presence of various bioactive components which include carbohydrate, protein, glycoside, steroids, alkaloids, flavonoids, lead acetate, tannin and phenolic compound, saponins were tabulated in Table 2. These phytochemicals present in the samples helps the body to regain its activity. Alkaloids have been associated with medical uses for centuries and their common biological property is their cytotoxicity[10]. It is also reported to have analgesic[11], antispasmodic and antibacterial properties[12,13]. Flavanoids have been associated with many of the biological effects such as antibacterial, antiviral, anti inflammatory, antiplatelet, antioxidant, free radical scavenging, vasodilatory actions etc [14].

Table 1: Chemical analysis of Cervilon and Lumbaton

S. No.	Parameters	Sample I (cervilon)	Sample II (lumbaton)
1	Acid value	0.224	0.337
2	Saponification value	53.3	43.8
3	Peroxide value	16.0	19.8
4	Iodine value	3.45	3.68

Table 2: Phytochemical constituents of Cervilon and Lumbaton

S. No.	Phytochemical test	Sample I	Sample II
I	Carbohydrate	present	present
Ii	Protein	present	present
Iii	Glycoside	Absent	Absent
Iv	Steroids [salkowsi test]	Absent	Absent

V	Alkaloids[hager's test]	Present	Present
Vi	Flavanoids [lead acetate test]	Present	Present
Vii	Tannin and phenolic[ferric chloride test]	Absent	Absent
Viii	Saponin [triterpens test]	present	present

Figure 1: Total bacterial count of sample I and II

SAMPLE (I) : CERVILON (15734) SAMPLE (II) : LUMBATON (16201)

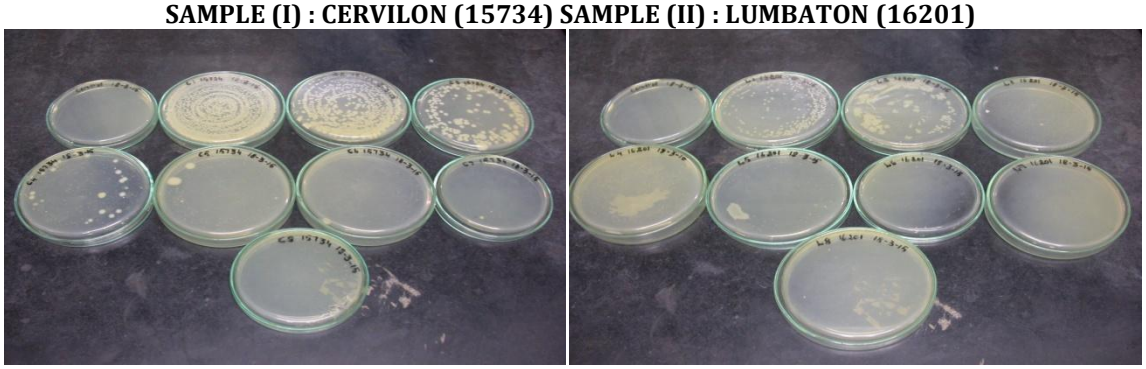


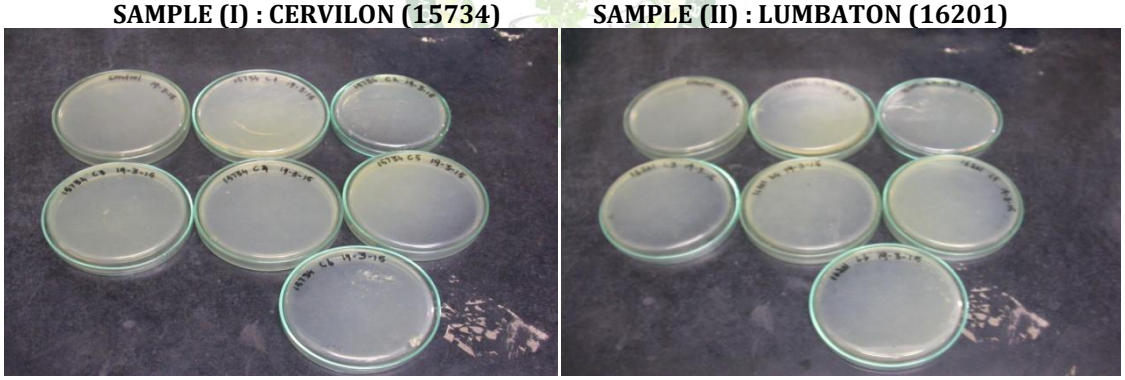
Table 3: Bacterial count on cervilon and lumbaton capsule

S. No.	Dilution	Cervilon	Lumbaton
1	Control	-	-
2	10^{-1}	Uncountable	Uncountable
3	10^{-2}	Uncountable	Uncountable
4	10^{-3}	Uncountable	Uncountable
5	10^{-4}	22	Uncountable
6	10^{-5}	4	38
7	10^{-6}	2	144
8	10^{-7}	1	5
9	10^{-8}	Nil	2

Many studies require the quantitative determination of bacterial population. It is a standard plate count method is an indirect measurement cell density and reveals information related only to live bacteria.

Figure 2: Total fungal count on sample I AND II

SAMPLE (I) : CERVILON (15734) SAMPLE (II) : LUMBATON (16201)



There is no fungal count was observed in plates

Antimicrobial activity

Antimicrobial activity was observed in soft gel capsules. The disease causing bacteria and fungi was identified and they detect the antimicrobial activity.

Figure 3: Antimicrobial activity of sample I and II against Selected pathogens

SAMPLE (I) : CERVILON (15734) SAMPLE (II) :LUMBATON (16201)

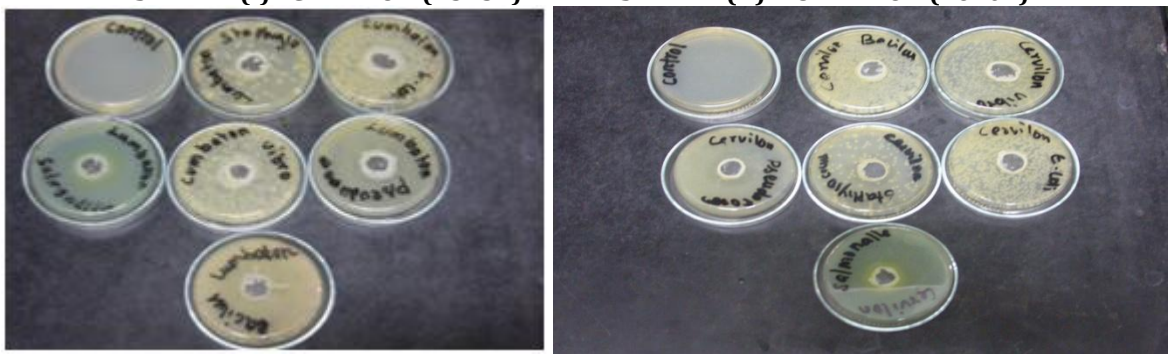


Table 4: Antimicrobial activity of sample (I) and sample (II)

S. No	Organisms	Sample I [15734] (cm)	Sample II [16201] (cm)
1	*Salmonalla typhi*	1.4	**Nil**
2	*Escherichia coli*	3.5	**3.2**
3	*Pseudomonos aerogens*	**1.0**	**1.2**
4	*Vibrio cholera*	**Nil**	**Nil**
5	*Staphylococcus aureus*	**1.4**	**Nil**
6	*Bacillus subtilus*	**Nil**	**Nil**

An antimicrobial activity of capsules of Cervilon and Lumbaton was observed by agar well diffusion method and by measuring the diameter of Zone of inhibition in (cm). Sample extracts was taken against 6 different organisms. Significant increase in the zone of inhibition was observed on increasing the concentration of extracts. Respective solvents were used as control.

High Performance Thin Layer Chromatography for extractions an its different fractions

HPTLC Profile:

Instrument used: CAMAG make HPTLC.

Software : winCATS 1.4.3

Sample Applicator: Linomat 5.

Detection : @254nm, @366nm and @520nm in Densitometry TLC

Scanner 3

Sample preparation: All samples dissolved in Corresponding solvents

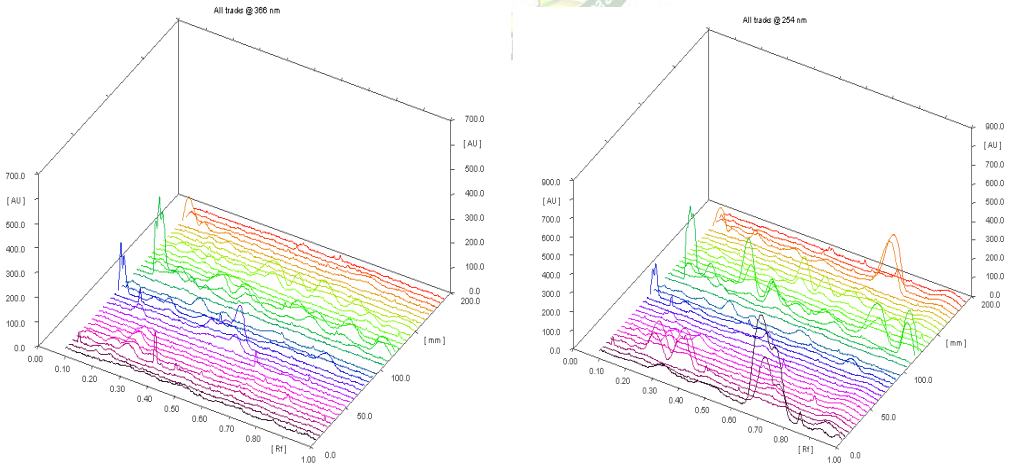
Stationary Phase: HPTLC plates silica gel 60 F 254

Mobile Phase : Toluene: Ethyl acetate (7.5:2.5)

Sample application: Cervilon, *Sida cordifolia, Aegle marmelos, Oroxylum indicum, Premna integrifolia, Tribulus terrestris, Stereospermum sauveolens, Gmelina arborea, Zizyphus jujube, Dolichos biflorus, Hordeum vulgare, Uraria picta, Desmodium gangeticum, Aerua lanata, Solanum melongena, Asparagus racemosus, Withania somnifera, Hemidesmus indicus, Tinospora cordifolia, Trigonella foenum-graceum, Boerhaavia diffusa, Acorus calamus, Cedrus deodara, valeriana wallichii, Pinus roxburghii, Rubia cordifolia, Glycyrrihiza glabra, Elettaria cardamomum, Phaseolus trilobus, Inula racemosa, Anethum sowa, Parmelia perlata, Mesua ferrea, Holostemma annulare, Ipomea digitata samples were applied as Track 1 to track 35 respectively.*

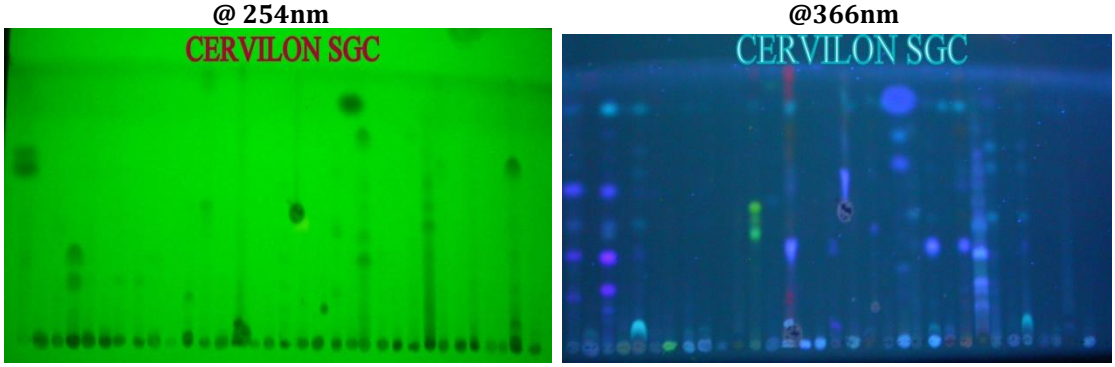
Fingerprinting: 3d Display

@ 254nm **@366nm**



Visualization

@ 254nm **@366nm**



Rf Value Tables:@254nm

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
0.20	0.18	0.10	0.12	0.14	0.20	0.14	0.16	0.12	0.13	0.23	0.20	0.45	0.17	0.10	0.67	0.10
0.31	0.29	0.18	0.17	0.25	0.26	0.18	0.26	0.26	0.34	0.93	0.35	0.51	0.24	0.21	0.95	0.17
0.46	0.32	0.37	0.25	0.35	0.34	0.26	0.67	0.35	0.66		0.55	0.55	0.73	0.26		0.25
0.52	0.69	0.47	0.36	0.52	0.54	0.67	0.78	0.43	0.78		0.93	0.93	0.96	0.39		0.35
0.69	0.75	0.58	0.44	0.69	0.65	0.69	0.94	0.59	0.92					0.45		0.44
0.75	0.93	0.76	0.46	0.91	0.68	0.78		0.67						0.50		0.62
0.84	0.94	0.93	0.50	0.93	0.83	0.80		0.77						0.66		0.77
0.93			0.68	0.98	0.92	0.92		0.91						0.71		0.83
			0.70		0.94			0.93						0.84		
			0.94													
20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	
0.16	0.23	0.13	0.12	0.35	0.19	0.12	0.16	0.22	0.23	0.27	0.39	0.11	0.10	0.13	0.48	
0.22	0.37	0.24	0.35	0.38	0.25	0.20	0.25	0.72	0.36	0.35	0.87	0.16	0.15	0.20	0.62	
0.28	0.46	0.29	0.44	0.67	0.44	0.28	0.37	0.76	0.68	0.68	0.93	0.24	0.16	0.25	0.94	
0.32	0.69	0.35	0.53	0.86	0.65	0.37	0.50	0.93	0.74	0.89	0.95	0.30	0.24	0.39		
0.49	0.80	0.44	0.64		0.85	0.44	0.58		0.94	0.94		0.39	0.38	0.47		
0.54	0.81	0.54	0.66		0.97	0.50	0.68					0.50	0.60	0.66		
0.67		0.60	0.84			0.58	0.79					0.75	0.75	0.76		
0.88		0.64				0.68	0.86					0.93	0.93	0.82		
		0.71				0.86	0.93					0.95	0.94	0.93		
		0.85				0.93										

@366nm

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
0.52	0.18	0.10	0.12	0.11	0.26	0.19	0.26	0.26	0.66	0.84	0.19	0.45	0.17	0.09	0.10	0.10
0.65	0.23	0.18	0.20	0.20	0.34	0.26	0.37		0.77		0.35	0.51	0.28	0.21	0.67	0.35
0.72	0.35	0.21	0.27	0.27	0.54	0.69	0.72		0.81		0.46	0.55	0.57	0.27		0.44
0.84		0.58	0.37	0.34	0.68	0.78	0.78				0.55		0.64	0.40		0.63
0.97		0.76	0.44	0.52	0.83									0.45		0.65
			0.48	0.69										0.50		0.77
			0.70	0.98										0.65		
20	21	0	23	24	25	0	27	28	29	30	31	0	33	34	35	
0.16	0.23	0.13	0.13	0.12	0.19	0.13	0.15	0.21	0.23	0.21	0.82	0.10	0.10	0.47	0.48	
0.22	0.27	0.22	0.36	0.21	0.26	0.20	0.21	0.23		0.27	0.87	0.16	0.46	0.66	0.59	
0.28	0.37	0.36	0.43	0.35	0.65	0.26	0.30	0.93		0.35		0.24	0.60		0.62	
0.33	0.46	0.44	0.64	0.38	0.85	0.39	0.36	0.96				0.40	0.76			
0.45	0.49	0.59	0.83		0.97	0.45	0.48					0.67	0.88			
0.49	0.62	0.64				0.50	0.58					0.76				
0.54	0.71	0.71				0.58	0.67									
0.65	0.80	0.83				0.68	0.79									
0.89	0.82					0.79	0.93									
						0.85										
						0.92										

High Performance Thin Layer Chromatography for extractions an its different fractions

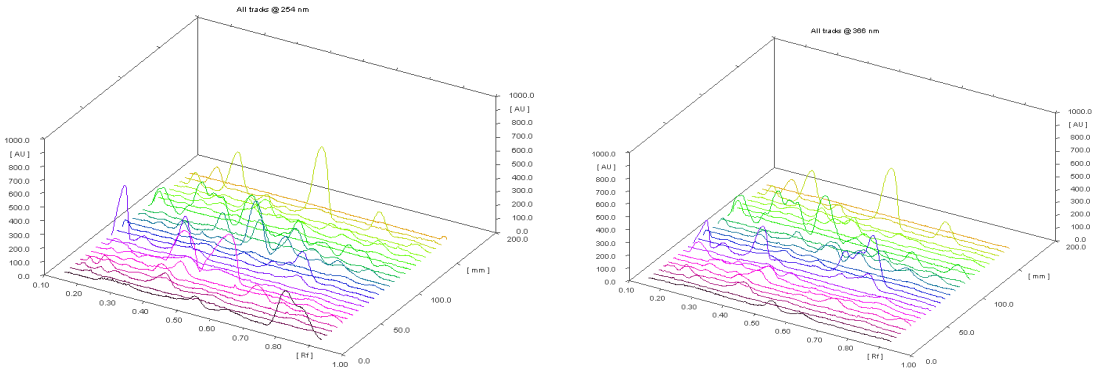
HPTLC Profile:

Instrument used : CAMAG make HPTLC.
Software : winCATS 1.4.3
Sample Applicator : Linomat 5.
Detection : @254nm, @366nm and @520nm in Densitometry TLC Scanner 3
Sample preparation : All samples dissolved in Corresponding solvents
Stationary Phase: HPTLC plates silica gel 60 F 254.
Mobile Phase : Toluene: Ethyl acetate (7.5:2.5)

Sample Application: Lumbaton, *Strobilanthes ciliatus, Gmelina arborea, Tribulus terrestris, Aegle marmelos, Stereospermum sauveolens, Oroxylum indicum, Aerua lanata, Solanum indicum, Uraria picta, Desmodium gangeticum, Premna integrifolia, Tinospora cordifolia, Cedrus deodara, Boerhaavia diffusa, Ricinus communis, Commiphora mukl, Cedrus deodara, Anethum sowa, Valeriana wallichii, Vetiveria zizanioides, Rubia cordifolia, Elettaria cardamomum, Callicarpa macrophylla, Coleuz vettiveroides* were applied as Track 1 to Track 23 respectively.

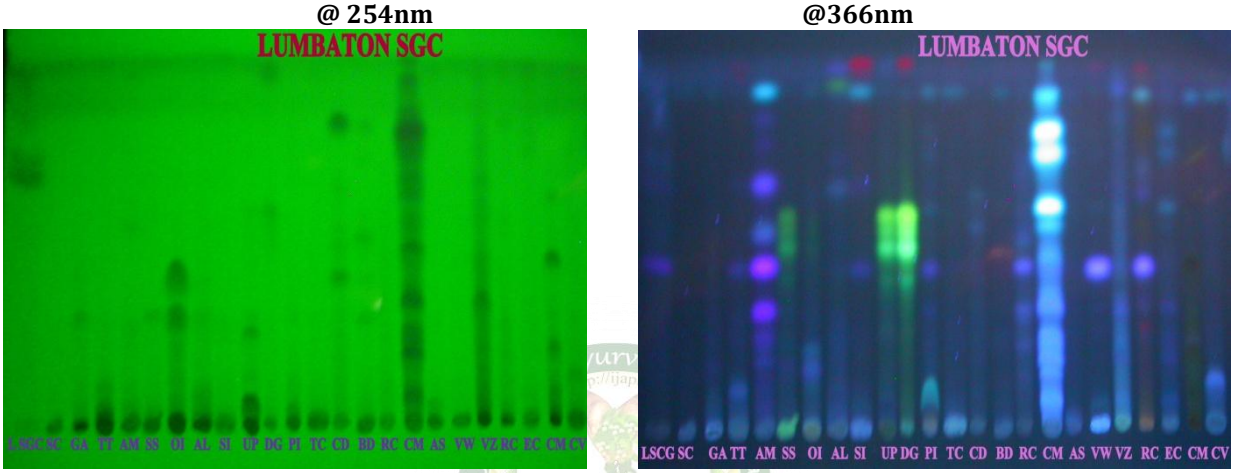
Fingerprinting: 3D Display

@ 254nm **@366nm**



Visualization

@ 254nm **@366nm**



Rf value Tables:@254nm

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
0.25		0.21	0.17	0.21	0.26	0.27	0.19	0.21	0.18	0.36	0.15	0.33	0.29	0.15	0.34	0.18	0.16	0.28	0.25	0.22	0.17	0.19	0.23
0.53		0.26	0.21	0.26	0.34	0.33	0.35	0.39	0.24	0.45	0.22	0.40	0.39	0.28	0.53	0.30	0.22	0.37	0.29	0.30	0.29	0.31	0.39
0.63		0.41	0.38	0.37	0.41	0.43	0.41		0.36	0.51	0.28	0.47	0.41	0.39	0.58	0.33		0.42	0.37	0.38	0.36	0.40	0.54
		0.55	0.41	0.56	0.61	0.78	0.48		0.40	0.61	0.40	0.78	0.52	0.51	0.65	0.36		0.55	0.44	0.47	0.44	0.57	
		0.80		0.67	0.68		0.77		0.61	0.66	0.50		0.60	0.60	0.72	0.45			0.49	0.56	0.51	0.74	
				0.79	0.70				0.70	0.70			0.76	0.67	0.80	0.55			0.86	0.63	0.85		
				0.88	0.79									0.75		0.73				0.68			
														0.83		0.88				0.79			
																				0.83			

@366nm

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
0.25	0.20	0.16	0.17	0.21	0.21	0.18	0.18	0.21	0.18	0.36	0.15	0.33	0.22	0.16
0.33	0.52	0.21	0.21	0.26	0.26	0.24	0.40	0.38	0.24	0.47	0.39	0.40	0.29	0.22
0.44	0.57	0.27	0.32	0.32	0.34	0.29	0.49	0.79	0.36	0.51	0.50	0.51	0.35	0.29
0.53		0.41	0.40	0.37	0.41	0.32	0.66		0.39	0.61	0.77	0.78	0.39	0.39
0.63		0.51	0.50	0.66	0.57	0.40	0.79		0.62	0.70		0.79	0.51	0.51
0.80		0.55	0.55	0.73	0.68	0.54			0.70	0.79		0.88	0.60	0.61
		0.80	0.65	0.88	0.70	0.78							0.70	0.67
			0.81		0.79	0.79							0.76	0.75
														0.84

16	17	18	19	20	21	22	23	24
0.19	0.18	0.16	0.22	0.19	0.22	0.20	0.19	0.23
0.33	0.30	0.38	0.28	0.25	0.29	0.29	0.31	0.39
0.53	0.33		0.37	0.31	0.38	0.43	0.40	0.54
0.58	0.36		0.43	0.44	0.47	0.51	0.46	
0.65	0.45		0.55	0.50	0.63	0.57	0.57	
0.72	0.59		0.79	0.55	0.68	0.87	0.74	

0.80	0.67			0.68				
	0.74			0.78				
	0.78			0.88				
	0.86							

The soft gel capsule Cervilon and Lumbaton HPTLC profile at 256nm and 366nm Rf values was calculated in graph representation and tables. HPTLC fingerprinting is a rational option to meet the need for more effective and powerful quality assessment of the traditional system of medicine throughout the world. It is a modern, rapid, accurate and simple tool for detecting the marker compounds in the plant sample[15]. The present study gives information regarding various phyto-constituents present in Cervilon and Lambaton when scanned at 254 and 366nm. The separated spots had different Rf values and the percentage areas. The HPTLC images indicate that all sample constituents were clearly separated without any tailing.

CONCULSION

Data suggested that capsule were consistent with various identity, quality, and purity parameters such as chemical parameters, phytochemical parameters, HPTLC profile, bacterial count, fungal count and antimicrobial analysis. Selected poly herbal capsule have passed through all the WHO parameters which were tested. So it can be concluded that use of capsule was safer and ready to use.

ACKNOWLEDGMENT

The authors are thankful to AVN Ayurveda formulations (P) Ltd. Madurai, Tamilnadu, India for providing necessary facilities to carry out this research work.

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Cite this article as:
Sudharameshwari.K, Mareeshwari.K, Singaravadivelu.R. Standardisation of Polyherbal Drugs-Cervilon and Lumbaton Capsules. International Journal of Ayurveda and Pharma Research. 2017;5(6):27-33.

Source of support: Nil, Conflict of interest: None Declared

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