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Standardisation of *Yogaraja guggulu* — An Ayurvedic polyherbal formulation

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Standardisation of the Ayurvedic medicine, *Yogaraja guggulu* has been achieved by following modern scientific quality control procedures both for the raw material and the finished product. The obtained values/ranges of physical and chemical parameters can be adopted to lay down new pharmacopoeial standards to be followed for traditional preparation of *Yogaraja guggulu* with batch-to-batch consistency. The phytochemical constituents found to be present in the raw material used for the preparation of *Yogaraja guggulu* possibly facilitate the desirable therapeutic efficacy of the medicinal formulation as a whole in ailments, and also could help in knowing the underlying mechanisms of pharmacological action.

Keywords: Ayurvedic formulation, *Guggulu*, *Yogaraja guggulu*, Drug stability, Drug standardization

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Guggulu is an exudates obtained in the form of oleogum resin from the stem of *Commiphora mukul* (Hook.ex Stocks) Engl., belonging to family Burseraceae. It is known to have analgesic, antiinflammatory and antihypercholesterolemic properties among others. *Guggulu* is the principal ingredient of several Ayurvedic formulations of medicines that are used for various ailments. Medicines, such as *Navaka guggulu*, *Vatari guggulu*, *Kaishora guggulu*, *Yogaraja guggulu* are traditionally used for obesity, body pain, skin disorders, neurological and musculoskeletal problems, respectively. From ancient times, *Yogaraja guggulu* (YRG), a polyherbal formulation, has been in use for *vata vyadhi*s (neurological, musculoskeletal disorders) in general practice, and in *sandhigata vata* (osteoarthritis) in particular[1]. Practitioners usually identify different herbs used in YRG according to Ayurvedic parameters. The preparation of YRG is based on traditional methods in accordance with the procedures given in classical texts like *Bhaishajya Ratnavali*. Due to lack of modern pharmacopoeial standards laid down and followed for processing of YRG, the medicine prepared using traditional methods may not have the desired quality and batch-to-batch consistency. Hence, there is a need for standardisation of YRG following scientific parameters including organoleptic characters, chemical analysis, chromatographic pattern and microbial screening.

The work was undertaken in the trust as part of a programme of testing and validation of the traditional practice of using the Ayurvedic medicine, YRG in the treatment of *sandhigata vata*. In this connection, standardization of YRG becomes imperative. Some work already exists on standardisation of YRG[2-4]. However, the current work deals with details following standardisation guidelines involving Good Manufacturing Practices (GMP) prescribed for preparation of Ayurvedic medicines[5]. Standardization guidelines to be followed for herbal products provided by World Health Organization (WHO), European Agency for the Evaluation of Medicinal Products (EMEA) and United States Pharmacopeias (USP) have also been considered. In the standardisation procedures followed in the work, certain in process tests have also been developed and performed.

Methodology

Yogaraja guggulu is a polyherbal formulation consisting of 29 ingredients (Table 1) in all. Specific morphological parts of the plants (herbs) are used in the formulation. The ingredients in the formulation, except *guggulu*, are taken one part each. The principal ingredient *guggulu* is taken (in purified form) in a

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Table 1 — Ingredients of *Yogaraja guggulu*

Sanskrit name	Plant name	Part used	Quantity
Chitraka	Plumbago zeylanica Linn.	Root	1 part
Pippalimoola	*Piper longum* Linn.	Root	1 part
Yavani	*Hyoscyamus niger* Linn.	Seed	1 part
Krishnajeeraka	*Nigella sativa* Linn.	Seed	1 part
Vidanga	*Embelia ribes* Burm. f.	Seed	1 part
Ajamoda	*Apium graveolens* Linn.	Seed	1 part
Jeeraka	*Cuminum cyminum* Linn.	Seed	1 part
Devadaru	*Cedrus deodar* (Roxb.) Loud.	Heart wood	1 part
Chavya	*Piper chaba* Hunter, non Blume.	Root	1 part
Ela	*Elettaria cardamomum* Maton	Fruit	1 part
Saindhavalavana	*Rock salt*	-	1 part
Kushta	Saussurea lappa *C.B.Clarke*	Root	1 part
Raasna	*Alpinia galangal* Willd.	Root	1 part
Gokshura	*Pedalium murex* Linn.	Seed	1 part
Dhanyaka	*Coriandrum sativum* Linn.	Seed	1 part
Haritaki	*Terminalia chebula* Retz.	Fruit	1 part
Vibhitaki	*Terminalia bellirica* Roxb.	Fruit	1 part
Amalaki	*Emblica officinalis* Gaertn.	Fruit	1 part
Musta	*Cyperus rotundus* Linn.	Root nodules	1 part
Shunthi	*Zinziber officinale* Rosc.	Stem	1 part
Maricha	*Piper nigrum* Linn.	Seed	1 part
Pippali	*Piper longum* Linn.	Fruit	1 part
Twak	*Cinnamomum zeylanicum* Breyn.(Blume.)	Bark	1 part
Usheera	*Vetiveria zizanioides* (Linn.) Nash	Root	1 part
Yavakshara	*Hordeum vulgare* Linn.	Plant ash	1 part
Talisapatra	*Taxus baccata* Linn.	Leaf	1 part
Patra	*Cinnamomum tamala* Nees & Eberm	Leaf	1 part
Guggulu	Commiphora *mukul* (Hook.ex Stocks) Engl.	Oleogum resin	27 parts
Ghrita	Cow ghee / Clarified butter	-	1 part
Herbs used in purification of *Guggulu*			
Amalaki	Emblica officinalis *Gaertn.*	Fruit	
Vibhitaki	*Terminalia bellirica* Roxb.	Fruit	
Haritaki	*Terminalia chebula* Retz.	Fruit	
Guduchi	*Tinospora cordifolia* (Willd.) Miers ex Hook.f. & Thoms.	Stem	

Note: The nomenclature for the herbal ingredients has been adopted from the Compendium[13]

quantity equal to the total quantity of the ingredients No 1 to 27. The raw material was procured from the local market and nearby forest, dried, and identified based on the Ayurvedic parameters *varna* (colour), *gandha* (odour), *ruchi* (taste), *aakruti* (shape) and *parimana* (size). The authenticity of the species of the procured herbs was checked and confirmed. Samples of the raw material were then examined for probable adulterants, which were found to be absent[6]. Also samples were examined for foreign matters, which were removed and the raw material was also identified[6,7]. Organoleptic evaluation through further identification of sensory characteristics like colour, odour, taste, shape, size, texture and fracture was done. In macromorphological evaluation, the plants were arranged according to their morphological characteristics. Identification of the correct part (for example, leaf) of the plant to be used was done so as to avoid the use of a possible similar looking part (for example, bract) of the plant. Microscopic evaluation and cytomorphological evaluation were not done as extensive phytochemical analysis was performed.

The plant material (including *guggulu*) was cleaned–physical cleaning by using a sterilized cloth duster to remove dust and by air blowing to remove minute sand particles. The material, as per individual requirements, was treated with water containing the antimicrobial agents potassium meta bisulphite (0.1%) and isopropyl alcohol (70%). It was then dried at 60^0C. Samples of the purified raw material were then considered for quality analysis in accordance with WHO guidelines for acceptance. Phytochemical constituents like tannins, alkaloids, glycosides, fixed oils, gums, resins, steroids, terpenes, flavonoids in each of the ingredients (except *ghrita*) of YRG were identified (Table 2) through qualitative chemical analysis. Thin layer chromatography (TLC) was done and Rf values were calculated. The raw material was assessed through quantitative analysis of standardisation parameters that included foreign organic matter; moisture content; water, alcohol, ether soluble extractive values; *p*H; total ash; and acid insoluble ash[8]. Their quantities were calculated and were found to be well within the available standard values/ranges. The test done for crude fiber was in accordance with the recommendation of the United States Pharmacopoeia (USP)[9]. Resin content was calculated for *guggulu.* In a polyherbal formulation like YRG consisting of a large number of herbal ingredients, although microbial screening could be done for the raw material, the same was considered and performed later for the finished product. The approved raw material was packed in sterilized air-tight polybags and plastic containers and stored in a cool place[1]. Hygienic conditions were maintained by regular disinfecting of the work areas and weekly fumigation[8].

Pulverization of herbal material

Twenty five herbal ingredients (excluding *guggulu*) involved in the formulation were dried at 60 ºC. Further, in accordance with Ayurvedic procedures, 3 of 25 herbal ingredients–dry ginger, cumin seeds, long pepper were roasted over a mild fire. Then, 25 herbal ingredients were individually pulverized and sieved (through 100 mesh) to obtain fine powders. Each of the powders was taken in equal quantities (by weight) and thoroughly mixed together to get a homogenous mixture, readied for further use.

Processing of *guggulu*

In addition to the cleaning and purification procedures used for *guggulu* along with the other ingredients, further purification procedures as recommended were adopted with the help of a decoction of *triphala* and *guduchi*, to get rid of minute impurities that are generally present in *guggulu*[1,10].

Preparation of *triphala* and *guduchi* decoction

Purification of guggulu was done with a decoction prepared with triphala (3 myrobalans; haritaki–*Terminalia chebula*, vibhitaki–*Terminalia bellirica* and amalaki–*Emblica officinalis*) and guduchi–Tinospora cordifolia (Table 1). The ratio (by weight) of guggulu: triphala: guduchi is 3:3:6. Guduchi was taken in fresh form, cleaned with distilled water and was purified with the antimicrobial agents, potassium meta bisulphite (0.1%) and isopropyl alcohol (70%). Microbial screening was done and the microbial content was found to be within the limits. Chemical analysis was done and phytochemical constituents were identified in guduchi (Table 2). TLC for guduchi was also done. Each of the triphala constituents was taken in an equal quantity in the form of coarse powders (40 mesh) to form triphala mixture, and added to (pounded) guduchi twice the quantity of triphala mixture. The resultant material was mixed in water (16 times the quantity of the material) that was then heated at a temperature of 70 ºC till $1/4^{th}$ of the original quantity remained. The liquid was allowed to cool for the sediment to settle and then filtered. The decoction thus obtained was

Table 2 — Phytochemical constituents in the ingredients of *Yogaraja guggulu* and *Guduchi*

Sanskrit name	Tannins	Alkaloids	Glycosides	Fixed oils	Gums	Resins	Mucilage	Proteins	Steroids	Terpenes	Flavonoids
Chitraka	+	+								+	+
Pippalimoola	+	+		+	+	+		+		+	
Yavani	+	+		+	+						
Krishna jeeraka	+		+	+		+	+	+		+	+
Vidanga	+	+		+	+	+				+	
Ajamoda	+	+		+	+	+	+			+	
Jeeraka	+			+	+	+	+	+		+	
Devadaru				+		+				+	
Chavya	+	+		+	+	+				+	
Ela	+			+	+			+		+	+
Saindhavalavana											
Kushta	+	+		+	+	+	+			+	+
Raasna	+	+		+	+	+				+	+
Gokshura	+	+		+	+	+					
Dhanyaka	+			+	+		+	+		+	
Haritaki	+				+	+					
Vibhitaki	+				+	+					
Amalaki	+				+						
Musta	+	+		+	+			+			+
Shunthi	+				+	+	+			+	
Maricha	+	+			+					+	
Pippali	+	+		+	+	+				+	
Twak	+	+			+	+	+			+	
Usheera											
Yavakshara											
Talisapatra	+	+		+	+	+	+				+
Patra	+	+			+					+	
Guggulu					+	+			+	+	
Guduchi	+	+					+	+	+		

\+ Present

Table 3 — In process tests (for *triphala* and *guduchi* decoction)

Parameter	* EV	B-1 OV	B-2 OV	B-3 OV	B-4 OV	B-5 OV	B-6 OV	B-7 OV	Mean ± SD
Specific gravity at 29 ^{0}C	1.00 to 1.03	1.02	1.02	1.02	1.02	1.02	1.02	1.02	1.02 ± 0.00
*p*H	3.5 to 4.0	3.67	3.75	3.65	3.68	3.70	3.74	3.66	3.69 ± 3.90 E-02
Total solids w/w (%)	26 to 28	26.80	27.44	27.89	27.22	26.68	27.56	27.04	27.23 ± 0.43

EV= Expected Value; OV = Obtained Value, B-1, B-2 … = Batch numbers

*Reasons for setting the ranges

Specific gravity: The medium used for extraction is water with specific gravity equal to 1. Since the extract contains active constituents that are not highly water soluble, specific gravity of the extract is expected to be slightly more than 1.

*p*H: The extract contains mainly acids and tannins, and so *p*H is expected to be acidic.

Total solids: This test is performed to check whether the process of extraction is complete or not. As it comes out, the solubility of all the ingredients, on the average, is indeed in the range 26-28%

subjected to certain in process tests. The obtained decoction was tested for specific gravity, *p*H and total solids, whose values were found to be within the set limits (Table 3). The purpose was to ensure that all the water-soluble constituents from *triphala* and *guduchi* got extracted into the decoction, which was considered standardised.

Shuddhi (purification) of guggulu

The physically cleaned *guggulu* (taken in a quantity equal to that of the *triphala* mixture) in raw form was mixed with the standardised decoction of *guduchi* and *triphala* for purification. The mixture was heated to 60-70ºC with continuous stirring so that *guggulu* mass got dissolved. (During the process, a small quantity of *ghrita* was added to prevent charring of the material). The resultant mixture was filtered through a thin cotton cloth. The material still remaining in the cloth was repeatedly treated with hot water and filtered, for completion of the filtration process. The filtrate obtained was decanted to get rid of any finer impurities. The resultant liquid was heated to remove the water content and for *guggulu* to remain in the form of a pasty material. At this stage, some amount of *ghrita* was added to this *guggulu* and heating continued till a semi-solid consistency was attained.

Kuttitha (pounded) guggulu

The *guggulu* of semi-solid consistency was repeatedly pounded in a mortar adding sufficient amount of *ghrita*, this time for making *kuttitha guggulu*[11].

Preparation of yogaraja guggulu

Saindhavalavana and *yavakshara* were powdered and added to the readied powder mixture of 25 herbal ingredients. The resultant mixture thus obtained was mixed in the *kuttitha guggulu* to get a whole mass. The whole mass was continuously pounded in a mortar by adding one part of *ghrita*, in small quantities and YRG of pill making consistency was obtained. Nearly uniform sized pills of YRG were made by hand and dried in an air drier, and further dried at 60ºC (not beyond 60ºC to prevent cracking) to remove excess moisture content. Pills were packed in amber colored, sterilised glass bottles that were labeled, coded and tightly closed with screw caps. The same were stored inside cool and dry shelves. Hygienic conditions were maintained. The detailed procedures were adopted for seven batches of YRG prepared. Statistical analysis was done; Mean and SD values are given (Tables 3 & 4); SE values were calculated. Range and Median values are mentioned wherever applicable.

Results

As part of standardisation procedure, the finished product *Yogaraja guggulu* was tested for relevant physical and chemical parameters and also subjected to microbial screening through quality control measures. Quality tests for the finished product (Table 4) were performed for the parameters moisture content, resin content, ash content and acid insoluble ash content and they were found to be close to or within standard ranges/values[5]. Also tests for *p*H, sulphated ash and crude fiber were done[8,9]. Further, tests for soluble extractive values in water, methanol, ether, ethyl acetate, hexane, chloroform and petroleum ether were done. It could be seen that the soluble extractive value (%) decreases as one moves down from water to petroleum ether. In addition, TLC was done (Fig. 1) with methanol extract of YRG[6]. A mixture of petroleum ether and ethyl acetate (3:1) was used as the mobile phase and iodine vapors as visualizing agent. Rf values were calculated and HPLC was also performed (Fig. 2). The finished product for contamination with heavy metals was got analysed by atomic absorption spectroscopy[7]. The values obtained for the tested heavy metals are: mercury (<0.1ppm); lead (6.5ppm); cadmium

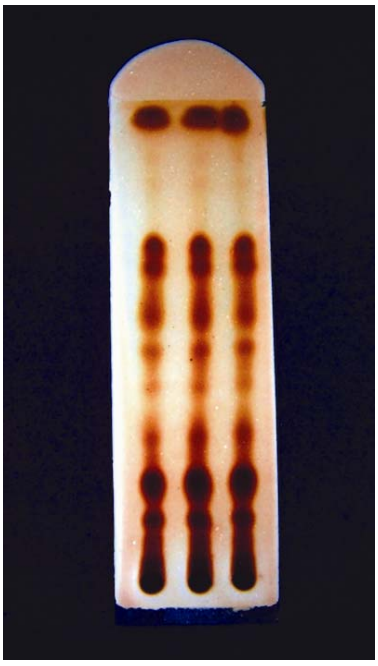

Fig. 1 — TLC for three consecutive batches of *Yogaraja guggulu*

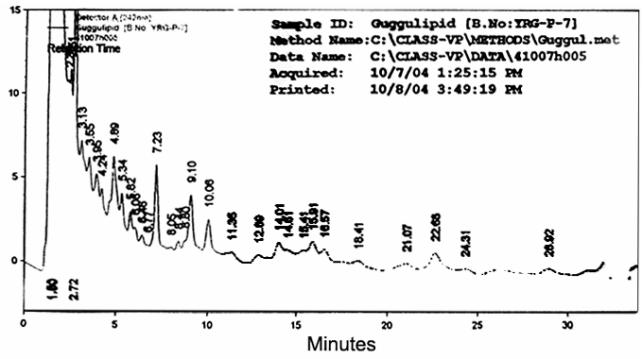

Fig. 2 — HPLC with retention times for a sample in a batch of *Yogaraja guggulu*

(<0.1ppm); arsenic (<0.2ppm). The values obtained are well within the acceptable limits, so that the finished product YRG is suitable on account of presence of the heavy metals within limits if it were to be prescribed to patients[12].

For the finished product, microbial analysis was done[6]. Pathogens, *E. coli*, *S. aureus*, *Salmonella*, *Shigella* and *P. aeruginosa* were found to be absent. Total aerobic count was done and bacteria (range 1125, median 975), fungi (yeast: range 1, median 0; moulds: range 10, median 8) and coliforms (range 5, median 2) were found to be within limits. Stability of the finished product was checked by testing for parameters moisture content, resin content, ash content and acid insoluble ash for a sample of YRG at two different times[5]. The results obtained (Table 5) were found to be within acceptable ranges/values and were nearly constant over the tested interval of time. Microbial screening for the finished product at different times showed that the counts for bacteria, fungi and coliforms were within the acceptable ranges as also absence of pathogens. Quality tests, microbial analysis and stability tests were done for all the batches of YRG prepared.

Discussion

The standardisation of *yogaraja guggulu* has become possible by considering various scientific parameters concerning the quality protocol while

Table 4 — Quality tests for the finished product *Yogaraja guggulu*

		Batch 5	Batch 6	Batch 7	Mean ± SD
Parameter	SV	OV	OV	OV	
Average pill weight (mg)	500	502	500	505	502.33 ± 2.51
Moisture content (%)	< 5	4.08	4.12	4.07	4.09 ± 3.00E-02
*Resin content (%)	5 to 10	4.33	4.28	4.12	4.24 ± 0.11
Ash content (%)	< 10	7.32	7.58	7.49	7.46 ± 0.13
Acid insoluble ash (%)	< 5	0.47	0.56	0.46	0.47 ± 5.50E-02
*p*H	-	4.19	4.22	4.18	4.19 ± 2.08E-02
Water soluble extractive (%)	-	35.05	34.96	35.12	35.04 ± 8.02E-02
Methanol soluble extractive (%)	-	32.30	32.38	32.24	32.30 ± 7.02E-02
Ether soluble extractive (%)	-	22.18	22.20	22.13	22.17 ± 3.61E-02
Ethyl acetate soluble extractive (%)	-	21.69	21.66	21.74	21.69 ± 4.04E-02
Hexane soluble extractive (%)	-	21.06	21.12	21.14	21.10 ± 4.16E-02
Chloroform soluble extractive (%)	-	20.77	20.63	20.86	20.75 ± 0.11
Petroleum ether soluble extractive (%)	-	20.73	20.41	20.53	20.64 ± 0.10
Crude fiber (%)	-	2.62	2.64	2.47	2.57 ± 9.29E-02
TLC (observed no of spots)	-	17	17	17	
Rf values (calculated)	0.03; 0.08; 0.13; 0.23; 0.32; 0.42; 0.49; 0.55; 0.57; 0.62; 0.67; 0.74; 0.79; 0.86; 0.91; 0.95; 1.00				

SV = Standard Value; OV = Obtained Value

* The obtained value is slightly less than the lower range of the standard value. This could be due to some of the resin content having been used up for interaction with the fat content in the *ghrita* used.

Table 5 — Stability tests for *Yogaraja guggulu*

Parameters	SV	YRG tested on 20/04/2003	YRG tested on 20/08/2004
		OV	OV
Average pill weight (mg)	500	505	502
Moisture content (%)	< 5	4.07	4.02
Resin content (%)	5 to 10	4.12	4.16
Ash content (%)	< 10	7.49	7.38
Acid insoluble ash (%)	< 5	0.46	0.58
*p*H	-	4.18	4.20
Water soluble extractive (%)	-	35.12	35.06
Methanol soluble extractive (%)	-	32.24	32.30
Ether soluble extractive (%)	-	22.13	22.18
Ethyl acetate soluble extractive (%)	-	21.74	21.62
Hexane soluble extractive (%)	-	21.14	21.04
Chloroform soluble extractive (%)	-	20.86	20.82
Petroleum ether soluble extractive (%)	-	20.53	20.41
Sulphated ash (%)	-	9.29	9.18
Crude fiber (%)	-	2.47	2.60
TLC (observed no of spots)	-	17	17

SV = Standard Value; OV = Obtained Value

keeping intact the procedures in accordance with Ayurvedic System of Medicine. A step that was followed during the processing of YRG was the pounding of *guggulu* done repeatedly with addition of small amounts of *ghrita* to make *kuttitha guggulu*. Also, continuous pounding of the powder mixture and *guggulu* mass together was done while adding one part of *ghrita* as an ingredient of the formulation to get the finished product YRG. The importance of repeated pounding could be to presumably facilitate synergy among the various active constituents in YRG and regulate their release inside the body, thereby enhancing absorption of the medicine. The *ghrita* used in the processing of YRG is supposed to minimise potential adverse effects (like gastric irritation) during digestion. While the various tests have their individual significance, tests done for different solvent extractives (Table 4) for the finished product have their own relative significance. The observation that the soluble extractive value (%) decreases as one moves down from water to petroleum ether could be attributed to the fact that decrease in solubility is as a result of the decrease in polarity of the solvents. It can also be inferred that appropriate processing sequence was adopted for preparation of the finished product. The possibility of orderly release and availability of the phytochemical constituents in the medicine in the gastrointestinal system is indicated.

For the parameters–*p*H, various soluble extractives, sulphated ash, crude fiber–for YRG, there are no standard ranges available. The Mean value obtained for each of these parameters was found to be consistent across the three considered batches with minimum SD. Further, the corresponding values of SE were calculated and found to be low. So, the inclusion of these parameters along with their respective obtained values could be considered for laying down new pharmacopoeial standards while preparing YRG according to traditional methods. The leading phytochemical constituents in YRG like tannins, alkaloids, glycosides, fixed oils, gums, resins, steroids, terpenes, flavonoids have pharmacological action on their own or in conjunction with body fluids, in terms of 'efficacy', to possibly help the body to help itself to reckon with ailments. To check expected batch-to-batch consistency as part of standardisation of YRG, recordings of TLC and HPLC were obtained for 3 consecutive batches. The occurrence of same number of spots in TLC plates (Fig.1) and the occurrence of relevant peaks at the same retention times in HPLC (shown in a manner of superimposibility in Fig. 3) confirms the consistency of the finished product YRG. Such a stipulation for obtaining TLC (including the number of spots and corresponding Rf values) and HPLC recordings could be considered and laid down as part of standardisation

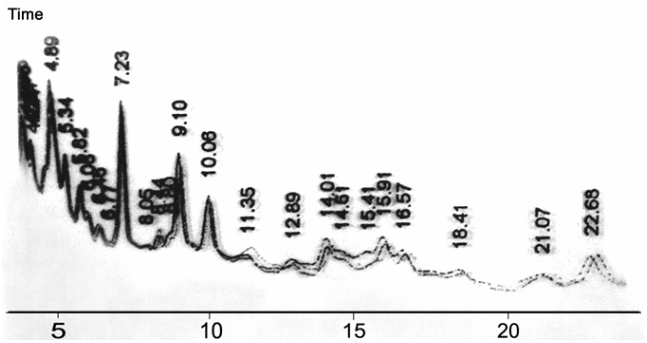

Fig. 3 HPLC with retention times for three consecutive batches of *Yogaraja guggulu* showing superimposibility

guidelines for preparation of YRG. The fact that the finished product YRG was found to be stable over a period of time of up to 16 months, by conducting tests for the various parameters, is more than indicative that the medicine has not lost its therapeutic value.

Conclusion

Ayurvedic medicine *yogaraja guggulu* has been standardised by intervention of modern scientific quality control measures in the traditional preparation described in classical texts. The results obtained could be used to lay down a new set of pharmacopoeial standards for the preparation of *yogaraja guggulu* for possible optimal efficacy of the medicine.

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