Standardization and Phyto-chemical Screening of a Unani Drug 'Berg-e-Sem' (*Dolichos lablab* L.) with Modern Analytical Techniques

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Abstract

Berg-e-Sem (*Dolichos lablab* L.), a Unani drug of repute has been standardized by modern techniques. The pharmacopoeial parameters studied include : physico-chemical, phyto-chemical, HPTLC, microbial load and aflatoxins etc. which reveal that it is safe for medicinal use. The study will serve as standard reference for quality check of the drug investigated.

Keywords: Barg-e-Sem, Standardization, Phyto-chemical screening, HPTLC.

Introduction

Standardization of herbal drugs direct towards the confirmation of its identity and determination of its quality and purity. At present due to advancement in the chemical knowledge of crude drugs, various methods like botanical, chemical, spectroscopic and biological are used for estimating active constituents present in the crude drugs. Every crude drug and herbal product is only as good as its quality. And highest quality standards are the key driver of success. Drugs must be marketed as safe and therapeutically active formulations whose performance is consistent and predictable. New and better medicinal agents are being produced at an accelerated rate. At the same time more exacting and sophisticated analytical methods are being developed for their evaluation. The medical fraternity at large is looking at alternatives from natural sources to combat diseases particularly those in which conventional modern system of medicine has little to offer. This realization on the one hand has increased demand for herbal drugs and on the other hand need for their quality standardization has gone up.

Barg-e-Sem, *Dolichos lablab* L. belongs to family Leguminosae. Synonyms: *Dolichos lablab* L.; *Dolichos purpureus* L.; *Lablab niger* Medikus; *Lablab lablab* (L.) Lyons; *Lablab vulgaris* (L.) Savi. An herbaceous, climbing, warm seasonal, annual or short-lived perennial herb with a vigorous taproot. It has a thick, herbaceous stem that can grow up to 3 feet, and the climbing vines stretching up to 25 ft from the plant (Valenzuela and Smith, 2002). It has trifoliate, long-stemmed leaves. Each egg-shaped leaflet widens in the middle and 3–6 in. (7.5–15 cm) long. The surface of the leaflet is smooth above and short haired below. Known as: Sanskrit-Nispava; Hindi-Sem, Bhatvas, Shimi; English-Lablab bean etc. Within India, *Lablab,* a field crop is mostly confined to the peninsular region and cultivated widely in Andhra Pradesh and adjoining districts of Tamil Nadu, Karnataka and Maharashtra.

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The global acceptance and interest in herbal medicines has led to an increase in their demand leading to a decline in their quality, primarily due to a lack of adequate regulations pertaining to these drugs (Rajini and Kanaki, 2008). WHO emphasized the necessity to ensure standards of medicinal plants drugs by using modern techniques (Imam *et al*., 2009; Rasheed *et al*., 2010a; 2010b; 2010c;2010d; 2011; 2012a; 2012b; 2012c; 2014a; 2014b; Naikodi *et al*., 2011). The hyacinth bean is an old domesticated pulse and multi-purpose crop *(Smartt and John, 1985; Shivashankar and Kulkarni, 1992).*

In order to overcome certain shortcomings of the pharmacopoeial monograph, other quality control measures must be addressed (Pifferi *et al*., 1999; Shinde, 2009; Singh and Soni, 2004; Street *et al*., 2008). The organoleptic parameters like taste, odour, colour etc. are not adequate to establish the standard quality of the medicine. Therefore, with a view to standardize and lay down the pharmacopoeial standards as per the standard operating procedures (SOP's), the leaf of the Unani drug *Dolichos lablab* was subjected to analysis for phyto-chemical, physico-chemical parameters, microbial load, aflatoxins, and high performance thin layer chromatographic studies. The paper presents the results of the studies carried out on this drug to ensure its quality for commercial use.

Materials and Methods

Collection of material

The leaf material of the *Dolichos lablab* L. (Barg-e-sem) was obtained from the locality or surrounding region of Hyderabad and was authenticated and identified by the botanist Dr. V.C. Gupta, at Central Research Institute of Unani Medicine, Hyderabad.

Chemical analysis

Physico-Chemical parameters of the *Dolichos lablab* was studied such as total ash, acid insoluble ash, solubility matter in alcohol and water, loss on drying at 105^0C, microbial load and aflatoxins contamination as per the methods described in WHO guidelines (Anonymous, 1998). Phytochemical screening was carried out in different solvents extracts such as Ethanol, methanol, Ethyl acetate, Chloroform, Petroleum ether, acetone and aqueous extracts as per the methods described by Trease and Evans (1972).

HPTLC analysis**:** DESAGA Sarstedt Gruppe system was used for analysis along with Automatic TLC applicator and UV visible cabinet as imaging system, the instrument had Proquant 1.6 version as software system for documentation.

Preparation of extract for HPTLC analysis

Five grams fine powder of dried Barg-e-sem was reflux on water bath for 30 min in Ethanol, Ethyl acetate, Chloroform and Petroleum ether (60-80^{0}C) separately through soxhlet apparatus. Later the contents were removed and filtered through Whattmann No. 41 filter paper and evaporated the solution to 20 ml. Thus the solution so obtained was used as sample for the determination of components.

Chromatography

HPTLC was carried out on the Precoated Aluminium Sheets of Silica Gel 60 F_{254} (Merck) dimensions of 20 cm × 10 cm. Different solvent extracts of about 10ìl were applied as 4 mm width bands using Automatic TLC applicator system of the DESAGA Sarstedt Gruppe (Germany). A Linear ascending development with Toluene: Ethyl acetate: methanol (6:3:1) (v/v) as mobile phase was carried out in a twin trough glass chamber previously saturated with mobile phase vapour for 20 min. at room temperature (25 ± 2°C). The development of solvent distance was 86 mm. After development plates were air- dried. Scanning was performed using densitometer of DESAGA Sarstedt Gruppe (Germany) under UV 254nm and 366nm and after derivatizing with anisaldehyde sulphuric acid and observed under visible range, at Rf values and operated by ProQuant 1.06 version software. The source of radiation was a deuterium lamp emitting a continuous UV spectrum in the range 190– 400 nm. The slit dimensions were 4 mm × 6mm.

Development of HPTLC technique

After the development, TLC plate was then removed dried completely and detected with under UV Cabinet system for detection of spots. Further it was scanned with the Densitometer CD60 of DESAGA Sarstedt Gruppe system under UV 254nm and 366nm and after derivatizing with anisaldehyde sulphuric acid and observed under visible range, at Rf values mentioned in the table as shown in the figure 2. A corresponding densitogram was then obtained in which peaks are appeared for the corresponding spots being detected in the densitometer while scanning and the peaks area under the curve corresponds to the concentration of the component in the sample for the concentration that applied on the TLC plate.

Results and Discussion

Organoleptic characters: The drug consists of leaves which are dark green colour, on drying changes to light green having characteristic smell (figure 1). The physico-chemical parameters data are given in table 1; total ash 8.85-9.00 gm%;

Table 1: Physico-chemical parameters of the compound formulation 'Barg-e-Sem'.

S.No.	Parameters	%w/w
1	Total Ash (%)	8.85-9.00 gm%
2	Acid insoluble ash (%)	0.67-0.89 gm%
3	Alcohol soluble matter (%w/w)	18.12-20.34 gm%
4	Water soluble matter(%w/w)	31.31-32.43 gm%
5	Loss of wt. on drying at 1050C.	5.67-5.89 gm%

Fig. 1: Barg-e-Sem; *Dolichos lablab* L.

acid insoluble 0.67-0.89 gm%, alcohol soluble matter 18.12-20.34 gm%, and water soluble matter 31.31-32.43 gm%; The moisture content i.e., Loss of weight on drying at 105^0C was also determined and found to be 5.67-5.89 gm%.

Phytochemical screening for phytoconstituents was carried out in the aqueous extract. This revealed the presence of carbohydrates, glycosides, tannins, flavonoids. Whereas in ethyl acetate and chloroform extract revealed the presence of proteins and tannins; in petroleum ether it reveals the presence of glycosides, proteins, tannins; in ethanol and acetone extracts revealed the presence of tannins only as shown in the table 3.

Powdered drug was screened for fluorescence characteristic with or without chemical treatment such as powder as such, powder treated with 1N NaOH in Methanol, powder treated with 1N NaOH in water, powder treated with 1N HCl, powder treated with 50% HNO3 aqueous, powder treated with 50% H_2SO_4 aqueous, powder treated with glacial acetic acid and the observations pertaining to their colour in daylight i.e, visible region and under ultra-violet light were noticed and are presented in the table 4. Fluorescence analysis of powdered drug

Table 2: TLC profile of alcoholic, ethyl acetate, chloroform and pet. ether extract of Barg-e-Sem along with R_f values. Mobile phase: Toluene: ethyl acetate: methanol (6:3:1)

Peak list & densitogram of Barg-e-Sem ethanolic extract at UV 366nm with Rf values					
Peak no	**Y-Pos**	**Area**	**Area (%)**	**Height**	**Rf values**
1	8.8	807.53	25.4	414.65	0.04
2	26.8	7.35	0.2	4.44	0.27
3	34.4	36.19	1.1	16.37	0.37
4	39.8	245.61	7.7	108.01	0.44
5	44.0	25.89	0.8	13.94	0.49
6	53.8	232.13	7.3	80.91	0.62
7	59.6	45.49	1.4	11.97	0.70
8	77.8	1776.23	55.9	326.01	0.93
Peak list & densitogram of *Barg-e-Sem* ethyl acetate extract at UV 366nm with Rf values					
Peak no	**Y-Pos**	**Area**	**Area (%)**	**Height**	**Rf values**
1	9.0	63.19	1.0	36.49	0.04
2	33.0	138.31	2.1	62.99	0.35
3	38.4	421.84	6.5	183.42	0.42
4	42.8	54.55	0.8	28.18	0.48
5	53.4	770.70	11.8	240.21	0.62
6	60.8	32.61	0.5	8.05	0.71
7	77.4	5035.45	77.3	892.98	0.93
Peak list & densitogram of *Barg-e-Sem* chloroform extract at UV 366nm with Rf values					
Peak no	**Y-Pos**	**Area**	**Area (%)**	**Height**	**Rf values**
1	9.2	98.83	1.8	56.74	0.04
2	32.8	64.48	1.2	33.62	0.35
3	38.0	343.60	6.2	147.23	0.42
4	42.2	41.30	0.7	22.45	0.47
5	52.8	804.14	14.4	233.69	0.61
6	67.8	25.68	0.5	13.14	0.80
7	73.2	1069.98	19.2	377.28	0.87
8	77.8	3125.04	56.1	768.67	0.93
Peak list & densitogram of *Barg-e-Sem* pet ether extract at UV 366nm with Rf values					
Peak no	**Y-Pos**	**Area**	**Area (%)**	**Height**	**Rf values**
1	9.2	18.12	1.0	13.93	0.04
2	52.6	188.04	10.5	71.89	0.61
3	59.4	21.90	1.2	6.79	0.69
4	77.6	1566.65	87.3	393.45	0.93

Table 3: Phytochemical screening of the nature of compounds present in different solvent extracts of Barg-e-Sem'.

S.No.	Phytoconstituent	Acetone ext.	ethanol ext.	Meth ext.	E.A. ext.	Pet. ether ext.	Aqueous ext.	$CHCl_3$ ext.
1.	Alkaloid	-	-	-	-	-	-	-
2.	Carbohydrates	-	-	-	-	-	+	-
3.	Fixed oil	-	-	-	-	-	-	-
4.	Glycosides	-	-	-	-	+	+	-
5.	Phenols	-	-	-	-	-	-	-
6.	Proteins	-	-	-	+	+	-	+
7.	Steroids	-	-	-	-	-	-	-
8.	Tannins	+	+	-	+	+	+	+
9.	Flavonoids	-	-	+	-	-	+	-
10.	Saponins	-	-	-	-	-	-	-

Table 4: Fluorescence analysis of powdered drug:

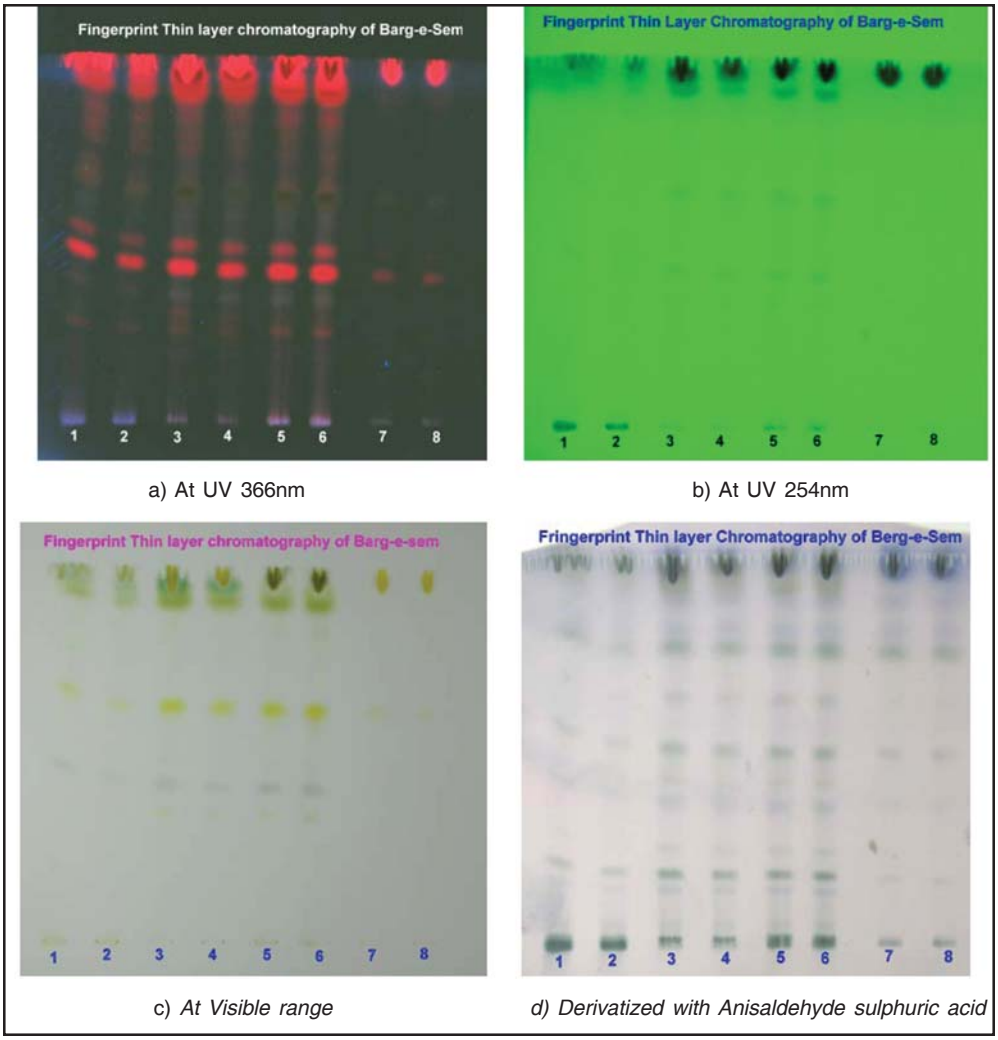
S.No.	Reagents	UV light		Visible light
		Short 254nm	Long 366nm	
1.	Powder as such	Black	Green	Green
2.	Powder treated with 1N NaOH in Methanol	Black	Pink	Green
3.	Powder treated with 1N NaOH in Water	Black	Reddish pink	Dark green
4.	Powder treated with 1N HCl	Black	Black	Dark brown
5.	Powder treated with 50% HNO3 aqueous	Black	Dark black	Brown
6.	Powder treated with 50% H_2SO_4 aqueous	Black	Black	Dark brown
7.	Powder treated with Glacial Acetic acid	Black	Reddish pink	Dirty green

extracts in seven different solvents was observed and has been reported in the table 5.

Safety evaluation of drug reveals that the total bacterial load and total fungal count of the microbial studies were found to be as 36 x 10^3/g and7 x 10

Table 5: Fluorescence analysis of powdered drug extracts in different solvents:

S.No.	Extraction Solvent	UV light		Visible light
		Short 254nm	Long 366nm	
1.	Acetone Extract	Black	Black	Dark green
2.	Alcoholic Extract	Black	Reddish pink	Dark green
3.	Chloroform Extract	Dark green	Pink	Dark green
4.	Petroleum ether extract	Black	Reddish pink	Light green
5.	Methanol	Dark green	Reddish brown	Dark green
6.	Ethyl Acetate	Black	Red	Dark green
7.	Distilled water	Dark brown	Dark green	Dark brown



a) At UV 366nm

b) At UV 254nm

c) *At Visible range*

d) Derivatized with Anisaldehyde sulphuric acid

Fig. 2: TLC chromatogram of *Barg-e-Sem* Ethanolic (Track 1,2); Ethyl acetate (Track 3,4); Chloroform (Track 5,6), Pet ether extract (Track 7,8) at UV 254nm and 366nm respectively. a) At UV 366nm, b) At UV 254nm, c) At Visible range d) At visible region after derivatizing with Anisaldehyde sulphuric acid reagent.

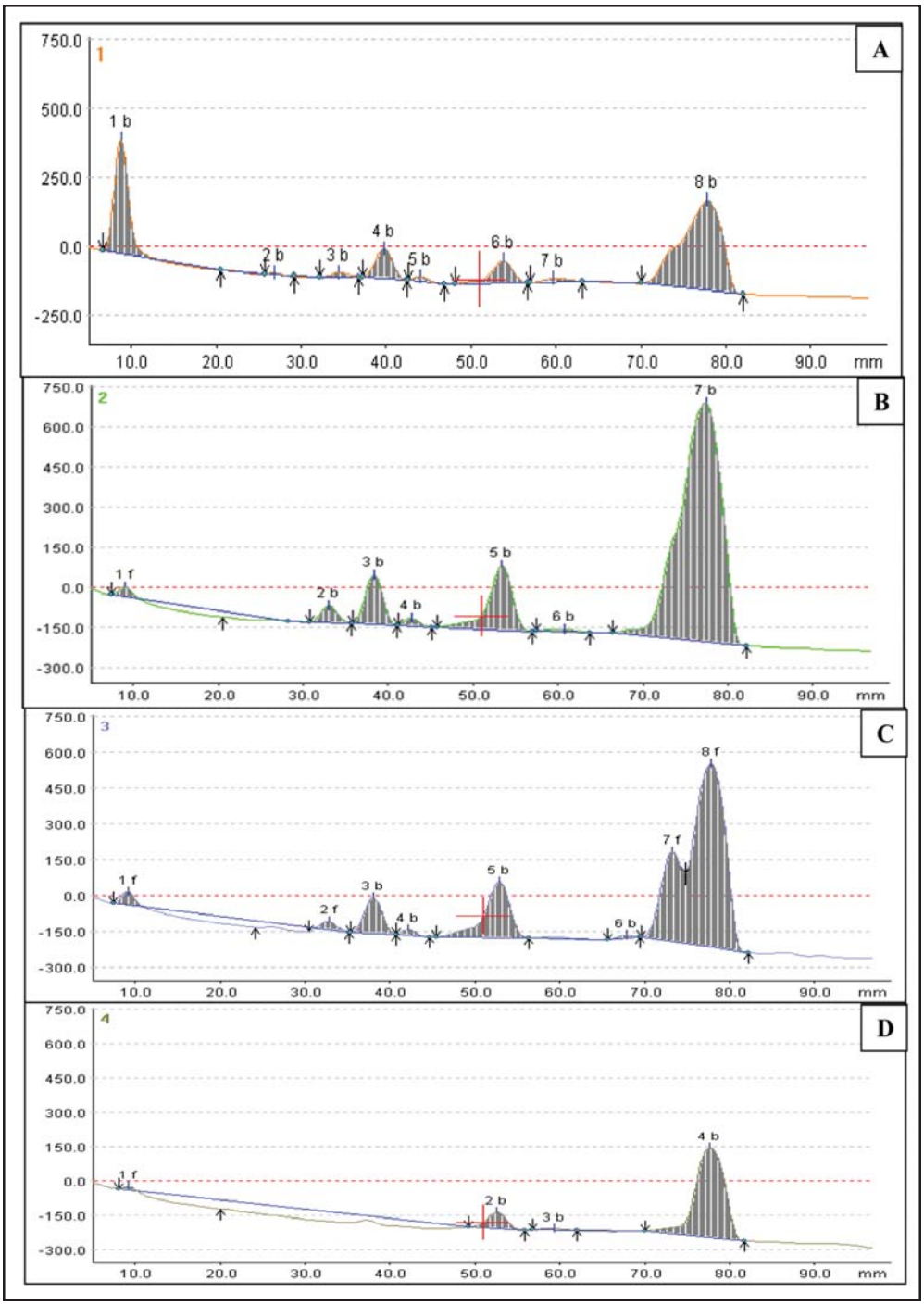


Fig. 3: HPTLC Densitogram of Barg-e-Sem at A) ethanolic extract B) ethyl acetate extract C) chloroform extract D) pet ether extract at UV 366nm

respectively which is within the permissible limits and the other parameters studied were found to be absent in the drug. The analysis of aflatoxins showed that the drug was free from any contaminations. These findings as observed for microbial load and aflatoxin contamination analysis are given in table 6.

Table 6: Aflatoxins, Microbial and fungal contamination in the drug.

Aflatoxin Contamination			
	Parameter analyzed	**Results**	**Permissible limits as per WHO**
1	B1	Nil	Not more than 0.50 ppm
2	B2	Nil	Not more than 0.10 ppm
3	G1	Nil	Not more than 0.50 ppm
4	G2	Nil	Not more than 0.10 ppm
Microbial and fungal Contamination			
	Parameter analyzed	**Results**	**Permissible limits as per WHO**
1	Total *Bacterial* Load	36 x 10^3	Not more than 10^5 / g
2	*Salmonella Spp*	Nil	Nil
3	*Escherichia Coli*	Nil	Nil
4	Total *Fungal* count	7 x 10	Not more than 10^3 / g

HPTLC analysis

HPTLC fingerprint studies of different solvent extract of Barg-e-sem was carried out and TLC plate developed and detected using the UV visible chamber which clearly showed various spots at UV 254nm and 366nm in the densitogram and also under visible region and after derivatizing with anisaldehyde sulphuric acid reagent. TLC profile of alcoholic, ethyl acetate, chloroform and petroleum ether extract of Barg-e-Sem along with R_f values under UV 366nm detection is illustrated in the table 2 and the corresponding HPTLC densitogram of Barg-e-Sem at A) ethanolic extract B) ethyl acetate extract C) chloroform extract D) pet ether extract was shown in figure 3. It was observed that there are eight spots in the Tlc plate of Barg-e-Sem Ethanolic extract at UV 366nm with Rf values 0.04, 0.27, 0.37, 0.44, 0.49, 0.62, 0.70, 0.93; it was observed that there are seven spots in the Tlc plate of Barg-e-Sem Ethyl acetate extract at UV 366nm with Rf values 0.04, 0.35, 0.42, 0.48, 0.62, 0.71, 0.93; it was observed that there are eight spots in the Tlc plate of Barg-e-Sem chloroform extract at UV 366nm with Rf values 0.04, 0.35, 0.42, 0.47, 0.61, 0.80, 0.87, 0.93; it was observed that there are four spots in the Tlc plate of Barg-e-Sem petroleum ether extract at UV 366nm with Rf values 0.04, 0.61, 0.69, 0.93. Thus established HPTLC fingerprinting profile will help to authenticate the leaves of the studied drug lablab and serve as a tool for the quality control analysis.

Conclusion

With a view to develop standard reference, the Unani drug Berg-e-Sem (*Dolichos lablab* L. - leaf) has been studied for physico-chemical, phytochemical screening,

HPTLC analysis and safety evaluation such as aflatoxins, contamination analysis, microbial load etc. which was found within permissible limits as per WHO guidelines. The data provided will serve as quality control standards for the drug investigated and help in the manufacture of genuine medicines and detect adulterations.

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