HPTLC Fingerprint Studies and Evaluation of Pharmacopoeial Standards for the Drug Habb-e-Sadar - A Unani Formulation

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Abstract

The use of natural products with therapeutic properties has been described by the practitioners of traditional medicines for several disorders. Habb-e-Sadar is a Unani formulation which is a combination of two single drugs namely, Momkham - bee wax and Mastagi - resin from *Pistacia lentiscus* L. It is the simplest formulation of one animal origin and one plant origin drug. As honey, the bee wax is also characterized by several therapeutic properties of great interest though not scientifically proven. Mastagi is also one of the widely used single drugs in Unani system of medicine without any proven scientific background. A very brief review of the literature on these two ingredients laid a way for standardisation of the drug Habb-e-Sadar, expecting that the synergistic effect of drug will result in exploiting many therapeutic properties for treating several ailments in future.

Keywords: Habb-e-Sadar Standardisation, Physicochemical analysis, TLC/ HPTLC analysis.

Introduction

The use of natural products with therapeutic properties is as ancient as human civilization. Therapeutic efficacy of many traditional classical products prepared from minerals, plants and animal have been prescribed by the practitioners of traditional medicines for curing several disorders. Habb-e-Sadar is one such Unani formulation prepared in the form of pills with the combination of two ingredients namely; Mom Kham (Bee Wax) and Mastagi Roomi – Resin (*Pistacia lentiscus* L.). It is the simplest combination of one animal origin drug (bee wax) and one plant origin drug (resin) in equal proportion. The drug is prescribed for treating Waj-ul-Sadar (Chest pain) patients and in some cases it is to be used as Musakkin (sedative) (NFUM, 2011).

Mom kham (bee's wax) is obtained by squeezing or pressing the honeycomb mostly produced by *Apis mellifera* or *Apis cerana*, after extraction of honey. It is a yellowish solid mass, harder than butter, has honey like odour, soluble in petroleum ether, slightly soluble in cold alcohol (3%), chloroform (25%) and insoluble in water. It is a complex mixture of hydrocarbons, free fatty acids, esters of fatty acids, diesters, exogenous substances like residues of propolis, pollen, small pieces of floral components and pollution. Generally the composition of the bees wax may vary between and among different families and different breeds of bees. Many medicinal properties of the bee wax have been known from ancient times. The "father of medicine", Hippocrates, recommended the use of bees wax in case of purulent tonsillitis (Filippo Fratini *et al.*, 2016; Pawan Kumar Sagar *et al.*, 2015). In Ayurveda, the bee wax is used under the name "Madhuchishtha"

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in treating wounds, burns and in curing heel cracks (Bagade Sarojini, 2013). Apart from that, Unani literature survey also reveals that bee wax is used in the treatment of various ailments like Sual-e-Yabis (Dry cough), Ishal-e-Muzmin (Chronic diarrhoea), Bawaseer (Haemorrhoids), Diq (Tuberculosis) and Humma (Fever). It is also used as one of the ingredients in the unani formulation Zimad Niswan.

The ingredient Mastagi Roomi is the resin obtained from the plant trunk *Pistacia lentiscus* L. (Family - Anacardiaceae). It is light yellow in colour with slight agreeable taste and fragrance. It is available in market as pear, ovoid or globular shaped small tears of 4-8 mm in diameter. The main constituents of the resin are α and β masticonic acids, α-masticoresene, β-masticoresene, α and β masticinic acids, volatile oil, masticolic acid, α-pinene and β-myrcene (Evans 1996 ; Wallis, 1997; Boelens and Jimenez, 1991). In Unani system of medicine, physicians are using mastagi since centuries for the treatment of many ailments like gastrointestinal disturbances, hepatobiliary disorders, gynaecological diseases, fractures, wounds and ENT problems (Shaikh Imtiyaz *et al.*, 2013). It is also used as one of the ingredients in many Unani formulations like Jawarish-e- Jalinoos, Jawarish-e-Mastagi, Jawahar Mohra and Habb-e- Ambar Momyai (NFUM, 2006 and 2008). The drug Habb-e-Sadar which is the combination of Momkham and Mastagi may be a right choice for further investigation of pharmacological activities, as the synergistic effect of drug will result in exploiting many therapeutic properties for treating several ailments. With this view, the study aimed to investigate the TLC/HPTLC fingerprint pattern of the drug with analysis of its physicochemical and quality control parameters to prove the scientific validation and to lay down pharmacopoeial standards for the drug Habb-e- Sadar.

Material and Methods

Collection of the Raw Drug and Preparation of the Formulation

Authentic raw drug Momkham (bee wax DSM-A-05) and Mastagi (resin DSM-149) were procured from Chennai market (R N Rajan Stores). The formulation Habb-e-Sadar was prepared in laboratory scale in the Drug Standardisation Research Unit, Regional Research Institute of Unani Medicine, Chennai, with utmost care by adopting good manufacturing practices as prescribed by the European Commission Brussels guidelines (2008) and NFUM (2011).

To the finely powdered mastagi, equal quantity of sliced, melted momkham was added and mixed thoroughly to obtain the lubdi mass. Manually the lubdi mass was rolled in between the fingers into sticks of required size, thickness and were cut into pieces using a knife. The cut pieces were further rolled to get the round shaped pills and stored in air tight containers. The drug was prepared in

three batches, each batch comprising a minimum of 500 pills weighting about 300 – 400 mg per pill.

Physico-chemical Analysis

The physico-chemical analysis viz., moisture content, extractive values, ash values and pH value were analysed for the prepared drug Habb-e-Sadar as per the standard methods (WHO, 2011)

Quality Control Analysis

Quality control parameters like microbial load, heavy metals, aflatoxins and pesticidal residues for the samples of Habb-e- Sadar drug were undertaken and analyzed. The microbial load estimation was carried out as per the guidelines (WHO, 2007). Heavy metal analysis was done by Atomic Absorption Spectrophotometer (AOAC, 2005). Analysis for aflatoxins was performed by TLC method (WHO, 2007). Pesticide residues were analysed using GC MS Agilent instrument equipped with mass selective detector as per the methods AOAC (2005).

TLC/HPTLC Fingerprint Analysis

The three batches sample of Habb-e-Sadar (each 5 gm) were extracted with 20 ml each of petroleum ether and chloroform separately and reflux on water bath for 30 mins and made up to 10 ml in a standard volumetric flask. The extract (5µl each) was applied over aluminium plate pre coated with silica gel 60 F_{254} (5 x 10 cm, E.Merck) by employing CAMAG ATS4 sample applicator. The plates were developed up to the distance of 8 cm in the chamber (10 x 10), using 10 ml of the developing system Toluene: Ethyl acetate (6: 4) as mobile phase for petroleum ether extract and 12 ml of Toluene : Ethyl acetate: Petroleum ether (9: 1: 2) for chloroform extract, dried at room temperature, observed and scanned under UV 254nm and 366nm. Finally, the plates were dipped in vanillin sulphuric acid reagent (200 ml) for a minute and heated at 105º C till coloured spots appear (Wagner and Bladt, 1984 and Sethi P D, 1996).

Results and Discussion

Physico-chemical Analysis

The physico-chemical standards for the drug Habb-e-Sadar is given in Table 1.

The pH and moisture content of the drug were found to be 7.4 and 0.394% respectively. Quantitative standards reveal the presence of negligible amount of siliceous matter in the sample where the total ash content was found to be 3.68% and acid insoluble ash was found to be 1.11%. The extractive value shows that the solubility of phytoconstituents of the drug was more in petroleum

ether (88.83%). Only negligible amount of phytoconstituents is soluble in alcohol (2.42%) and water (0.13%) when compared to petroleum ether. It is inferred from the data that the drug contains more fat soluble phytoconstituents that are soluble in non polar solvents.

Quality Control Analysis

A. Microbial Load

The microbial content of the sample is given in Table 2.

The estimation of microbial load gives the tentative idea to assess the quality and safety of the drug prepared. The assessment done for estimating the total viable count of bacteria, total fungal count, count of bacteria belonging to the Enterobacteriaceae family, count of pathogens like Escherichia coli, Staphylococcus aureus and Salmonella spp. indicates that the microbial load to be within the permissible limits of WHO stating that the drug is safe for internal use for the treatment of prescribed ailments.

B. Heavy Metal Analysis

The amount of various heavy metals found in the sample is given in Table 2.

Heavy metals are hazardous to human and animal health, their content in any drug used for consumption or medicinal purposes must be limited. Heavy metal contained in Habb-e-Sadar was found to be within the permissible limits of Ayurvedic Pharmacopoeia of India (API) and Unani Pharmacopoeia of India (UPI) stating that the drug is safer from toxic substances point of view.

C. Detection of Aflatoxins

Results of aflatoxin content in the sample tested are given in Table 2.

Aflatoxins are toxic metabolites produced by a variety of molds such as Aspergillus flavus, Aspergillus parasiticus and Aspergillus nomius. Reports of the present study do not show any evidence for the presence of any of the aflatoxin content (B_1, B_2, G_1, G_2) in the sample.

D. Detection of Pesticide Residues

The pesticide residue content of the sample is given in Table 3.

Production of herbal drugs according to good agricultural practices with no pesticide residues is highly uncontrollable due to several factors. Detection of pesticide in the samples also became a major task though several techniques have been developed. In the present study the pesticide residue was analysed using the GC-MS instrument which has the detection limit up to 0.01 ppm. The results suggested that the sample is free from pesticides.

TLC/ HPTLC Fingerprint Analysis

A. TLC/HPTLC of Chloroform Extract

The suitable mobile phase Toluene: Ethyl acetate: Petroleum ether (9: 1: 2) with appropriate proportion has been determined for chloroform extract of the drug Habb-e-Sadar. The TLC photographs of chloroform extract are shown in (Figure-1).

B. HPTLC Fingerprint of Chloroform Extract

HPTLC fingerprint profile of chloroform extract of Habb-e-Sadar showed 14 peaks (Figure-2). The densitometric chromatograms of three batch sample of the drug Habb-e-Sadar are recorded at 254 nm (Figure-3).

A. TLC/HPTLC of Petroleum ether Extract

The suitable mobile phase Toluene: Ethyl acetate (6: 4) with appropriate proportion has been determined for petroleum ether extract of the drug Habb-e-Sadar. The TLC photographs of petroleum ether extract are shown (Figure-4).

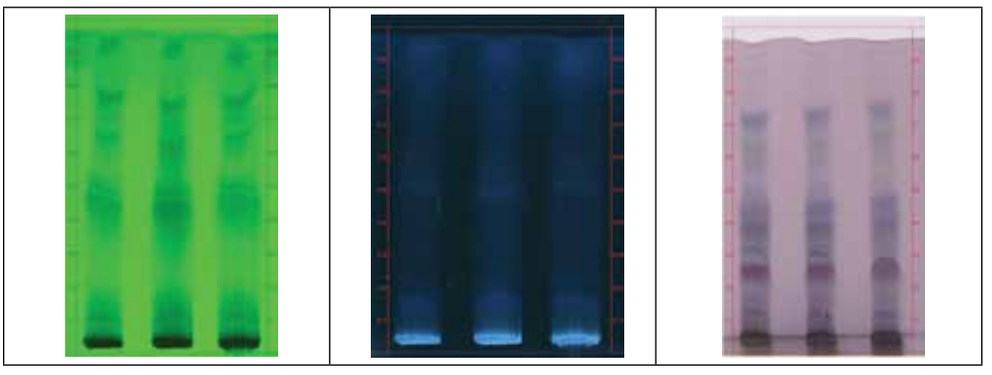
B. HPTLC Fingerprint of Petroleum ether Extract

HPTLC fingerprint profile of petroleum ether extract of Habb-e-Sadar showed 10 peaks (Figure-5). The densitometric chromatograms of three batch sample of the drug Habb-e-Sadar are recorded at 254 nm (Figure-6).

Conclusion

Up on investigation of the literature survey, the single drug mom kham and mastagi are found to exhibit numerous pharmacological activities as stated above though no work has proved scientifically. The drug Habb-e-Sadar, the combination of these two single drugs will be an appropriate choice to carry out many future pharmacological studies. The present approach for standardisation of the drug Habb-e-Sadar will serve as available scientific standards.

Chloroform extract



Solvent System: Toluene : Ethyl acetate : Petroleum ether (9:1:2)
Track 1. Batch - I; Track 2. Batch - II; Track 3. Batch - III

Rf values

UV 254nm	UV 366nm	VS Dipped
0.91 (Green)	0.90 (Blue)	0.76 (Blue)
0.78 (Green)	0.50 (Blue)	0.74 (Light blue)
0.71 (Green)	0.42 (Violet)	0.68 (Yellowish green)
0.68 (Green)	0.29 (Violet)	0.61 (Light violet)
0.51 (Green)	0.21 (Blue)	0.52 (Light violet)
0.41 (Green)	0.18 (Blue)	0.40 (Violet)
0.10 (Green)		0.32 (Blue)
		0.28 (Pink)
		0.20 (Light yellow)
		0.19 (Light blue)
		0.12 (Violet)

Fig. 1 : HPTLC photographs of Habb-e-Sadar

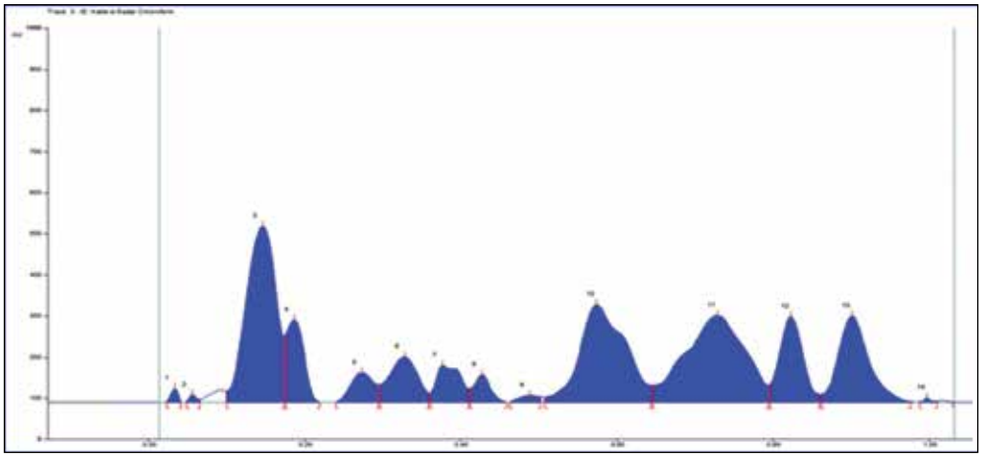


Fig. 2 : HPTLC fingerprint of Habb-e-Sadar chloroform extract at 254nm

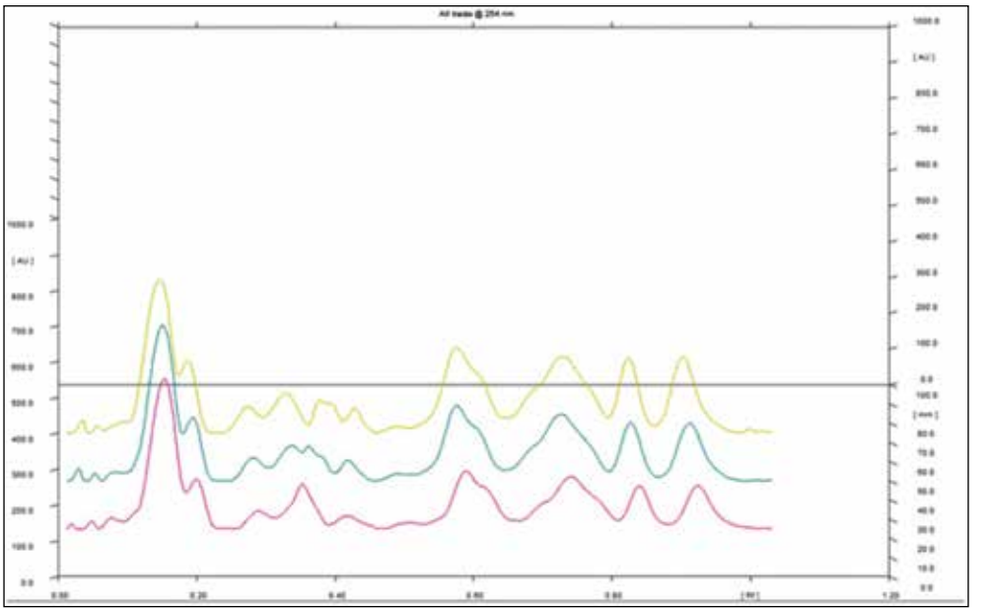
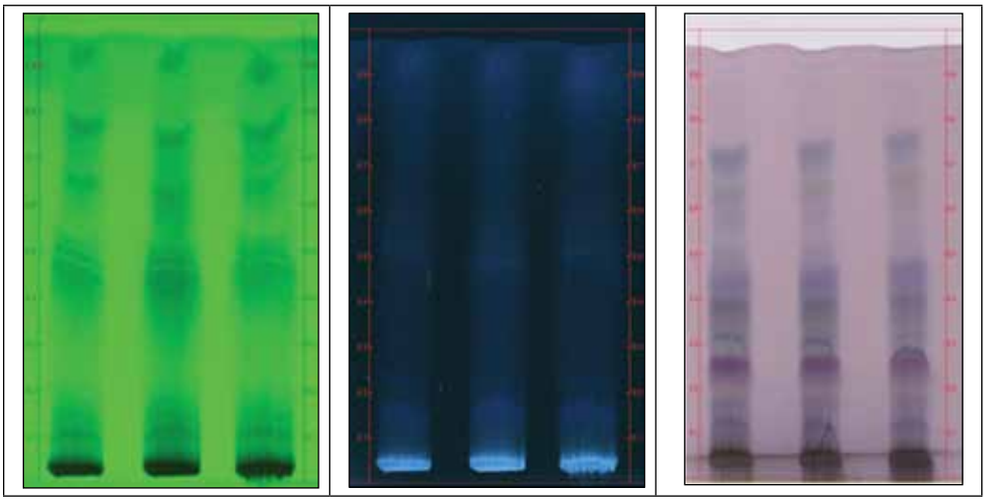


Fig. 3 : Densitometric chromatograms of Habb-e-Sadar chloroform extract at 254nm

Petroleum ether extract



Solvent System: Toluene : Ethyl acetate (6 : 4)

Track 1. Batch - I; Track 2. Batch - II; Track 3. Batch - III

Rf values

UV254nm	UV 366nm	VS Dipped
0.75 (Green)	0.91 (Violet)	0.90 (Violet)
0.70 (Green)	0.70 (Blue)	0.71 (Grey)
0.68 (Green)	0.59 (Blue)	0.65 (Yellowish green)
0.48 (Green)	0.55 (Blue)	0.59 (Light violet)

0.39 (Green)	0.51 (Blue)	0.50 (Light violet)
0.35 (Green)	0.48 (Blue)	0.46 (Violet)
0.34 (Green)	0.35 (Blue)	0.41 (Dark violet)
	0.31 (Blue)	0.38 (Yellowish green)
	0.18 (Blue)	0.32 (Violet)
	0.11 (Violet)	0.30 (Yellow)
		0.28 (Pink)
		0.17 (Light violet)
		0.10 (Violet)

Fig. 4 : HPTLC Photographs of Habb-e-Sadar

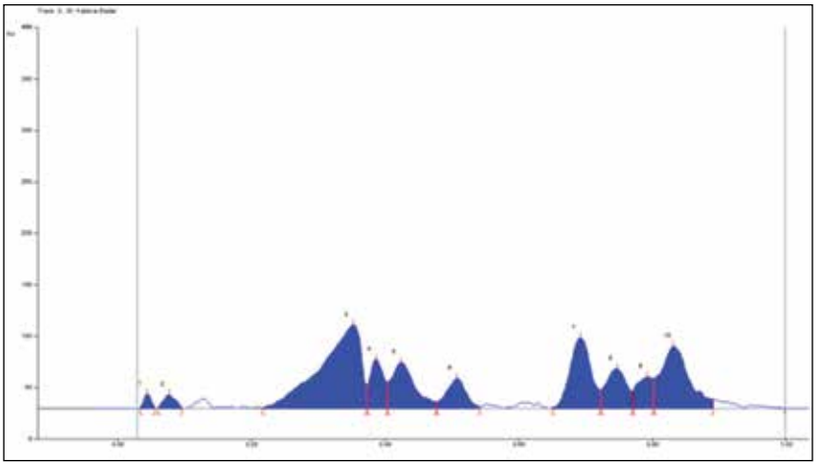


Fig. 5 : HPTLC fingerprint of Habb-e-Sadar Petroleum ether extract at 254nm

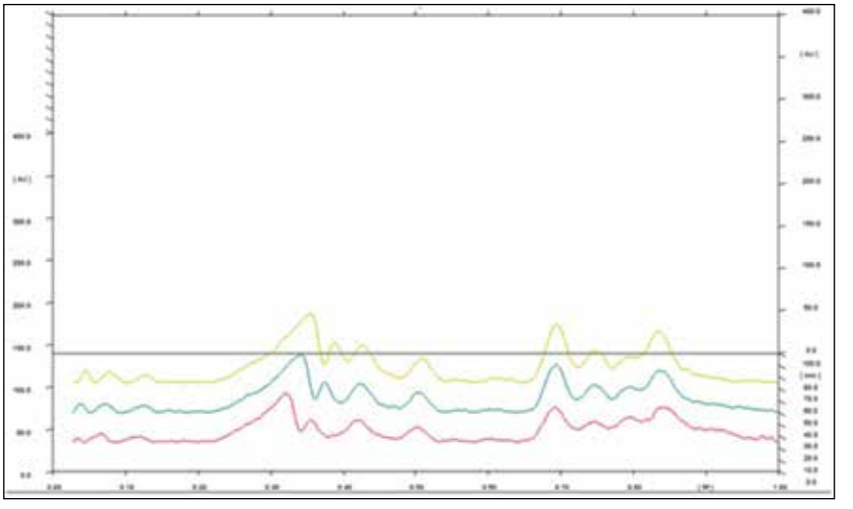


Fig. 6 : Densitometric Chromatograms of Habb-e-Sadar Petroleum ether extract at 254nm

Table 1: Physico-chemical Analysis of Habb-e-Sadar

Parameter Analysed	Batch Number		
	I	II	III
Extractive values			
Petroleum ether	88.86%	88.76%	88.87%
Alcohol	2.30%	2.46%	2.52%
Water	0.14%	0.13%	0.13%
Ash value			
Total Ash	3.69%	3.68%	3.69%
Acid insoluble ash	1.00%	1.13%	1.20%
Moisture	0.451%	0.375%	0.357%
pH Values			
1% solution	7.5	7.4	7.4
10% solution	5.2	5.2	5.1

Table 2 : Quality Control Analysis of Habb-e-Sadar

S. No.	Name of the Analysis	Parameter	Results	Permissible Limits
1.	Microbial Load	Total bacterial content	< 10 cfu/gram	$x10^5$ cfu/gm
		Total fungal content	<10 cfu /gram	$x10^3$ cfu/gm
		Enterobacteriaceae	Absent	Absent
		Escherichia coli	Absent	Absent
		Salmonella spp.	Absent	Absent
		Staphylococcus aureus	Absent	Absent
Permissible limits: World Health Organisation (WHO), 2007; Cfu/gm: Colony forming units per gram.				
2.	Heavy metals	**Name of the element**	**Results**	**Permissible Limits (ppm)**
		Lead	ND	10
		Cadmium	ND	0.3
		Mercury	ND	1
		Arsenic	ND	3

Permissible limits: The Ayurvedic Pharmacopoeia of India (API), 2008; The Unani Pharmacopoeia of India (UPI), 2016; ND: Not Detected; ppm: parts per million				
3.	**Aflatoxin**	**Parameters**	**Results**	**Inference**
		B1	ND	Absent
		B2	ND	Absent
		G1	ND	Absent
		G2	ND	Absent
ND: Not Detected				

Table 3: Pesticide Residue of Habb-e-Sadar

S. No.	Name of the pesticide compound	Results
1	DDT (all isomers, sum of ρ, ρ'-DDT, α, ρ' DDT, ρ, ρ'-DDE and ρ, ρ'-TDE (DDD expressed as DDT)	Not detected
2	HCH (sum of all isomers)	Not detected
3	Endosulphan (all isomers)	Not detected
4	Azinphos-methyl	Not detected
5	Alachlor	Not detected
6	Aldrin (Aldrin and dieldrin combined expressed as dieldrin)	Not detected
7	Chlordane (cis & trans)	Not detected
8	Chlorfenvinphos	Not detected
9	Heptachlor (sum of heptachlor and heptachlor epoxide expressed as heptachlor)	Not detected
10	Endrin	Not detected
11	Ethion	Not detected
12	Chlorpyrifos	Not detected
13	Chlorpyrifos-methyl	Not detected
14	Parathion methyl	Not detected
15	Malathion	Not detected
16	Parathion	Not detected
17	Diazinon	Not detected
18	Dichlorvos	Not detected
19	Methamidophos	Not detected

20	Phosalone	Not detected
21	Fenvalerate	Not detected
22	Cypermethrin (including other mixtures of constituent isomers sum of isomers)	Not detected
23	Fenitrothion	Not detected
24	Deltamethrin	Not detected
25	Permethrin (sum of isomers)	Not detected
26	Pirimiphos methyl	Not detected

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सारांश

हब्ब-ए-सदर-एक यूनानी मिश्रण का एच.पी.टी.एल.सी. अगुलांक अध्ययन एवं भेषजकोशीय मापदंड का मूल्यांकन

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चिकित्सीय गुणों से भरपूर प्राकृतिक उत्पादों का उपयोग पारंपरिक औषधियों के चिकित्सकों द्वारा बहुत सारी बिमारियों के ईलाज में किया जाता है। हब्ब–ए–सदर एक यूनानी मिश्रण है जिसे दो एकल औषधियों मोमखाम–मधुमक्खी के मोम और मस्तगी–पिश्तेसिया लेंटिस्कस एल की राल के मिश्रण से बनाया जाता है। यह एक पशु और पौधे के मूल से विकसित औषधि का सरलतम रूप है। शहद अर्थात् मधुमक्खी का मोम वैज्ञानिक तरीके से सिद्ध नहीं होने के बावजूद भी मधु के समान भरपूर चिकित्सीय गुणों से युक्त होता है। मस्तगी भी बिना किसी सिद्ध वैज्ञानिक सबूत के यूनानी चिकित्सा पद्धति में व्यापक रूप से इस्तेमाल की जाने वाली एकल औषधियों में से एक है। इन दो घटकों पर साहित्य की एक बहुत संक्षिप्त समीक्षा ने हब्ब–ए–सदर औषधि के मानकीकरण के लिए एक तरीका निकाला। दवा हब्ब–ए–सदर के सहक्रियात्मक प्रभाव से यह उम्मीद की जाती है कि भविष्य में यह औषधि कई बीमारियों के लिए कारगर सिद्ध होगी।