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Quality Evaluation and Pectolinarigenin Contents Analysis of Harak Remedy in Thailand

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ABSTRACT

Introduction: Harak remedy is a Thai traditional medicine for anti-pyretic treatment. Some researchers reported that crude drugs and capsules of Harak remedy, which were distributed throughout Thailand, have been adulterated with the upper ground parts. Moreover, there is no recent report of quality control of the Harak products after marketed. **Objective:** Thus, aim of our research was to investigate the quality of marketed Harak capsules following requirements of Thai Herbal Pharmacopoeia (THP). **Methods:** The hierarchical cluster analysis (HCA) and principal component analysis (PCA) reported the similarity of samples to authentic Harak. The 18 samples were purchased from 6 regions of Thailand, 3 samples in each region (HR01-HR18). The authentic plants were collected from Surin province and provided as capsule drug (HR19). In addition, the powder of authentic remedy was extracted by aqueous and ethanol then the chemical constituents were analyzed by GC-MS. **Results:** As the result of standard specifications, 15 samples were standard medicines (83.33%) while 3 samples had high levels of total aerobic bacteria, total yeast, and molds. The chemical fingerprint and quantification of pectolinarigenin of 19 samples were investigated by TLC and RP-HPLC. The pectolinarigenin content of HR19 was 25.3±0.31 mg/g drug powder and the most correlated with were 3 samples as HR08, HR10, and HR16. **Conclusion:** According to HCA and PCA, most of the samples showed similar data patterns except HR17. The result provides essential information for identification of the Harak remedy for the purpose of quality control.

Keywords
Harak, Quality evaluation, Pectolinarigenin, RP-HPLC

Introduction

Harak remedy is a Thai traditional herbal recipe which Thai traditional doctors have prescribed for the treatment of pyretic symptom in both children and adults. The remedy is recorded in a Thai scripture called Tak-Ka-Si-La as an antipyretic herbal drug. Nowadays, the remedy is registered as the National List of Essential Medicines A.D. 2013 of Thailand (List of Herbal Medicinal Products) by National Drug Committee. The remedy has several names such as Ben-Cha-Lo-Ka-Wi-Chian, Keaw-Ha-Duang and Petch-Sa-Wang. The recipe consists of five herbal roots in equal proportion by weight as follow *Harrisonia perforata* (Khon-Thaa: HP), *Ficus racemosa* (Ma-Dueo-Chumporn; FR), *Capparis micracantha* (Ching-Chee; CM), *Clerodendrum petasites* (Mai-Tao-Yai-Mom; CP), and *Tiliacora triandra* (Ya-Nang; TT).

Numerical pharmacological activities supporting the indication of this remedy have been reported. The mixed of roots powder showed antipyretic efficacy by using Baker's yeast-induced fever in a rat model [1]. In addition, all doses of mixed powder significantly (p-value<0.05) attenuated the increased rectal temperature produced by lipopolysaccharide (LPS) injection as potent as acetylsalicylic acid, positive control [2]. Furthermore, The ethanolic extract of Harak remedy possessed the highest nitric oxide (NO) inhibitory activity on the release of inhibitory activities against LPS in RAW 264.7 cell lines with an IC_{50} value of 40.4 µg/ml, which was lower than Indomethacin (IC_{50}=20.32 µg/ml), and the ethanolic extract of

Clerodendrum petasites, *Harrisonia perforata*, *Tiliacora triandra* and *Capparis micracantha* showed moderate inhibition activity (IC_{50}<60 μg/ml), while the *Ficus racemosa* extract showed no inhibition activity (IC_{50}>100 μg/ml) [3]. The observation of the traditional medicine market discovered that Harak ingredients have been adulterated with the upper ground parts [4]. As the same results, the sample of crude drugs and capsules distributed throughout Thailand were also stem adulteration [5]. Individual plants had many reports of their constituents for example; bergenin, triterpenes polypodatetraene,
α-amyrin acetate, gluanol acetate, lupeol acetate, beta-sitosterol, cycloartenol, and euphorbol from barks and roots of *F. racemosea* [6,7]; aromatic glycoside from roots of *C. micracantha* [8]; hispidulin, taraxerol, lupeol, 22-dehydroclerosterol, stigmasterol from roots of *C. petasites* [9]; tiliacolinine, tiliacorine and nortiliacorinine A from roots of *T. triandra* [9]; peucenin-7-methyl ether, O-methylalloptaeroxylin, perforamone A-D, pectolinarigenin, perforatinolone, harrisotone A-E, haperforine A-E, harrisonol A, harperforatin, harperfolide and harperamone from branches, stems, leaves, fruits and roots of *H. perforata* [11−14].

The ethanolic of Harak extract was found pectolinarigenin and O-metyllaloptaeroxyrin which were related to *H. perforata* based on its chemical constituents. Two compounds exhibited anti-allergic activity [15]. In addition, pectolinarigenin was isolated from several plants as *Millingtonia Hortensis* [16], *C. setidens* [17], *Hemistepta lyrata* [18] and including *Clerodendron siphonenthus* which is belongs to family VERBANACEAE as same as *C. petasites* [19]. Method validation for determine pectolinagenin in Harak extract by a reversed-phase high performance liquid chromatography (RP-HPLC) were evaluated, the content of pectolinarigenin was 18.50 mg/g of extract [20]. However, there are some reports of standard specifications of Thai herbal medicine remedy such as Hom-na-wa-khot, Hom-in-ta-juck, Chan-ta-lee-la and Leung-pid-sa-mud for consumer's safety [21], but there has not been studied on standard specifications and quality control of Marketed Harak remedy. Thus, the present research investigated quality of Marketed Harak Remedy in Thailand which were followed Thai Herbal Pharmacopoeia [22, 23] such as organoleptic, loss on drying, ethanol soluble extractive value, water soluble extractive value, total aerobic bacteria, total yeast and molds, total ash, acid-insoluble ash. The chemical constituents were inspected by GC-MS and TLC, the quantitative analysis of pectolinarigenin was studied by HPLC technique. Then, the statistical analysis also examined.

Methods

Samples

Harak remedy has generally marketed throughout Thailand as capsule which is the most common dosage form. Eighteen samples of the Harak capsules were purchased from 6 regions of Thailand. Total 18 samples (3 samples per region) were named as HR01-HR18. Only 8 samples of 18 samples were officially registered by FDA Thailand (Table1). All samples were stored at room temperature and avoided exposure to light and moisture until further use.

Preparation of authentic Harak remedy

Roots of five plant species were collected from Surin province in 2017 for using as authentic Harak remedy (HR19). The voucher specimens were deposited at the herbarium of Southern Center of Thai Medicinal Plants at Faculty of Pharmaceutical Science, Prince of Songkla University, Songkhla, Thailand. The roots of five plant species were authenticated by specialist and compare with Reference herbarium as follows: *Harrisonia perforata* (SKP 178081601), *Ficus racemosa* (SKP 117061801), *Capparis micracantha* (SKP 391031301), *Clerodendrum petasites* (SKP 202030901) and *Tiliacora triandra* (SKP 114202001). All plants were cleaned and dried at 45−50°C before combining in equal proportion (by weight) and ground as coarse powder. The powder was refined using 100 mesh sieves then the fine powder was compressed into capsule number 1 and packed in aluminum foil. Each capsule contains 250 mg of Harak remedy powder. The authentic capsule was stored at room temperature and avoided exposure to light and moisture until further use.

Analysis of chemical constituents in the authentic Harak remedy by using Gas Chromatography - Mass Spectrometry (GC-MS)

The coarse powder was divided into two parts. The mixed powder was boiled in water, filtered through Whatman No. 1 filter paper and dried by lyophillizer to obtain water extract. In the second part, the powder was macerated in 95% ethanol for 3 days, filtered then repeated 3 times and evaporated to obtain ethanolic extract. The percentage yield of the water and ethanolic extracts were 6.68% and 2.86%, respectively. Each extract was dissolved in methanol to produce 10 mg/ml of sample solution before filtered through 0.45 microliters nylon membrane filter.

GC-MS analyses of the aqueous and ethanolic extracts were carried out by using Thermo Focus GC (Thermo®, USA). One microliter of the extract solution was injected into GC system with split ratio of 1:50. The injector temperature was set at 200ºC. The chemical components of extract were separated along a Thermo® TG-5silms column (30 m x 0.25 mm x 0.25 micron). Column oven temperature was started at temperature of 60°C and raised to 300°C by temperature increment of 5°C /min. The interface temperature was set at 275°C.

Mass spectrum was detected by using scan mode from 100 to 500 m/z. Compounds presented in GC-MS chromatogram were identified by comparing to NIST libraries.

Evaluation of quality of Harak capsules

Quality of Harak capsules were evaluated following requirements of THP including description organoleptic, loss on drying, ethanol soluble extractive value, water soluble extractive value, total aerobic bacteria, total yeast and molds, total ash, acid-insoluble ash [22]. Moreover, chemical profiles of Harak capsule was investigated by thin layer chromatography (TLC) and high-performance liquid chromatography (HPLC). An important marker, pectolinarigenin, which expressed potent anti-inflammatory related antipyretic was determine quantitatively by a validated HPLC method of previous research [20].

Quantitative HPLC analysis of pectolinarigenin and HPLC fingerprint of Harak capsules

Chemicals and reagents

Standard pectolinarigenin (Purity>98%) was purchased from ChemFaces (Wuhan, China). HPLC reagents such as acetronitrile, methanol and phosphoric acid were purchased from RCI Labscan (Bangkok, Thailand). Purified water was prepared by Milli Q® system from Millipore (Bedford, MA, USA).

Preparation of samples for HPLC analysis

Powder from Harak capsules was accurately weighed for 200 mg and dissolved in 5 ml methanol. The sample mixture was sonicated for 15 minutes and filtered through a 0.45-micron membrane filter. The filtrated (20 mL) was injected into the HPLC system.

Preparation of standard solutions

The stock solution of pectolinarigenin was prepared by diluting the accurate weight of standard pectolinarigenin in methanol to produce concentration of 1.0 mg/mL. The working standard solutions were prepared by serially diluted the stock solution to produce concentration 0.1, 0.4, 0.8, 1.6, 2.4 and 3.2 µg/mL. The standard curve was constructed by plotted between concentration and peak area of the serial working standard solutions.

HPLC system

Studies of chemical fingerprint and quantitative analysis of active compound were carried out using our previous validated HPLC method described by of previous research [20]. HPLC system (Agilent® 1200, USA) composed of a quaternary pump (G1311A), photodiode array detector (G1315D) and automatic injector (G1329A). A reversed-phase column was ZORBAX Eclipse XDB-C18 column (4.6 x 250 mm, 5 micron) protected by Eclipse XDB-C18 analytical guard cartridge (4.6 x 12.5 mm, 5 micron). The mobile phase was a mixture of 0.1% ortho-phosphoric acid (A) and acetronitrile (B). Gradient elution was programmed as follows: 0–30 min: 95%A–5%A, 30–35 min: 5%A and 35–40 min: 95%A. The flow rate was 1 mL/minute with detection at UV 331 nm. The operating temperature was maintained at room temperature (25°C). Data were analyzed by ChemStation® software.

Data analysis

The similarity of HPLC fingerprints of Harak from difference sources were analyzed by using the hierarchical cluster analysis (HCA) and principal component analysis (PCA). Peak areas of common peaks found in chromatogram were subjected to the analyses. Heat map and HCA were performed by using Heatmap Illustrator (HemI) version 1.0 software [24]. PCA was performed by using MATLAB software (MathWorks®, USA).

Results

Physical appearances of sample and authentic Harak

The 18 samples were purchased from 6 regions including Central, Northern, Northeast, Western, Eastern and Southern part of Thailand. Only 8 samples of 18 samples were officially registered by FDA Thailand (Table 1). All samples contained in capsule number 1. The appearance of authentic powder (HR19) performed light brown color as same as samples except HR17 showing red color.

Table 1 The sources of manufacturing and drug registration of Harak capsules

Sample codes	The sources of manufacturing (provinces)	Regions	Drug registration
HR01	Nakhon Pathom	Central	Registered medicine
HR02	Nakhon Pathom	Central	-
HR03	Bangkok	Central	Registered medicine
HR04	Chiang Mai	North	-
HR05	Chiang Mai	North	-
HR06	Roi Et	Northeast	Registered medicine
HR07	Sakon Nakhon	Northeast	-
HR08	Buriram	Northeast	-
HR09	Trang	Southern	-
HR10	Tak	Western	-
HR11	Prachinburi	Eastern	Registered medicine
HR12	Prachinburi	Eastern	Registered medicine
HR13	Chachoengsao	Eastern	Registered medicine
HR14	Prachuap Khiri Khan	Western	-
HR15	Phetchaburi	Western	Registered medicine
HR16	Phatthalung	Southern	Registered medicine
HR17	Trang	Southern	-
HR18	Phitsanulok	North	-
HR19	Authentic Harak capsule		

Chemical constituents of authentic Harak remedy by GC-MS

There are 18 constituents found in the aqueous extract of Harak remedy consisting of 14 compounds and 4 unknown compounds (Table 2). The ethanolic

extract performed 31 compounds and 4 unknowns compounds (Table 3).

Table 2 The chemical and unknown constituents of aqueous extract of authentic Harak remedy

RT	Text name	% Area
13.75	2-Naphthalenol	15.17
15.95	3-Nonanol	7.98
22.86	1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-HEXADECAMETHYL-OCTASILOXANE	4.80
23.71	2,4-Di-tert-butylphenol	2.12
25.65	Cedrenol	2.91
26.01	Unknown 1	6.91
26.70	Cyclooctasiloxane, hexadecamethyl	10.48
27.75	Methyl linolelaidate	2.65
30.01	Cyclononasiloxane, octadecamethyl	8.57
31.48	Lucenin	1.19
32.38	7,9-di-tert-butyl-1-oxaspiro[4.5]deca-6,9-diene-2,8-dione	8.78
33.49	Isopropyl Myristate	2.96
35.30	Unknown 2	1.44
36.12	Unknown 3	2.69
36.61	Stearic acid	3.19
36.78	Unknown 4	4.20
37.27	9,12,15-Octadecatrienoic acid	7.06
40.80	1,3,5-CYCLOHEPTATRIEN,7,7-DIMETHYL-2,4-DIPHENYL	6.89

Quality of samples

The quality control according to THP showed that 15 of 18 samples passed criteria of the requirements (83.33%). Three samples (16.66%) failed the requirements of total aerobic bacteria and total yeast and molds which were higher than standard level. The results of standard specifications of HR01-HR19 shown in Table 4. The TLC fingerprint represented the chemical compounds of samples which showed in three different solvent systems order of increasing polarity. HR17 which showed red powder performed the most different pattern when compare to the others (Figure 1).

Pectolinarigenin quantification of samples

HPLC fingerprint and determination of pectolinarigenin were conducted according to our previous validated HPLC method [20]. The calibration curve of standard pectolinarigenin is shown in figure (2). Retention Time (RT) of pectolinarigenin was 20.9 mins, which was a small peak whereas some peaks were higher than pectolinarigenin peak (Figure 3). Content of pectolinarigenin constituted in HR19 was 25.3±0.31 mg/g of drug powder. HR08, HR10 and HR16 showed the most equal % content to the authentic HR19 while the content pectolinarigenin in HR15 and HR17 cannot be detected (Table 5).

Table 3 The chemical and unknown constituents of ethanolic extract of authentic Harak remedy

RT	Text name	% Area
13.89	Camphor	0.85
14.96	Terpinene-4-ol	0.16
15.97	2-Hexanol	0.32
16.38	Laurine	0.15
19.79	Eugenol	0.18
20.46	Junipene	0.10
21.10	n-Docosane	0.08
21.59	Isolongifolene	0.20
21.80	(-)-Spathulenol	0.12
21.89	Isoledene	1.12
22.04	1(10),4-aromedenedradiene	0.20
22.50	Nipagin	0.04
25.43	Strophanthidin	0.05
25.56	Caryophyllene oxide	0.68
25.66	Azulene	0.61
25.96	n-Dotriacontane	0.60
27.36	Curcumene	0.29
29.46	2-Propen-1-one, 1-cyclohexyl	0.50
30.83	Cembrene	0.09
32.19	Sclarene	0.19
32.38	7,9-di-tert-butyl-1-oxaspiro[4.5]deca-6,9-diene-2,8-dione	0.20
33.57	Palmitic acid	0.91
34.16	Palmitic acid ethyl ester	4.82
36.94	Methyl linoleate	2.52
37.21	Linoleic acid	11.12
37.35	Oleic acid	32.50
37.73	17-Octadecen-14-yn-1-ol	23.99
37.82	Stearic acid	6.42
40.77	Hydroxynaphthazepinetrione	2.52
42.84	Unknown 1	0.52
44.27	Methyl tricosanoate	0.35
47.16	Unknown 2	0.25
47.39	Unknown 3	0.41
55.67	Unknown 4	1.35
56.45	Lupeol acetate	5.59

Table 4 The results of standard specifications of samples

Sample	% Total ash	% Acid-insoluble Ash ± SD	% Ethanol soluble Extractive value ± SD (N=3)	% Water soluble Extractive value ± SD (N=3)	% Loss on Drying ± SD (N=3)	Total aerobic microbial (CFU/g)	Total yeasts and molds (CFU/g)
HR01	4.43	0.48	2.515 ± 0.312	6.770 ± 0.077	6.51 ± 2.97	<10	<10
HR02	4.10	0.40	3.557 ± 0.041	7.908 ± 0.569	6.19 ± 2.82	<10	<10
HR03	4.03	0.44	2.780 ± 0.142	7.737 ± 0.179	8.75 ± 4.19	2.2×10^5	**$8.5 \times 10^{3***}$**
HR04	4.98	0.72	5.599 ± 0.079	7.687 ± 0.905	7.00 ± 2.90	2.1×10^4	2.0×10^1
HR05	3.92	0.48	5.200 ± 0.078	6.888 ± 0.449	9.74 ± 4.20	2.5×10^5	1.1×10^3
HR06	4.85	1.05	5.278 ± 0.094	9.125 ± 0.013	8.36 ± 3.46	<10	<10
HR07	4.46	0.60	3.869 ± 0.076	10.956 ± 0.039	6.84 ± 2.66	<10	<10
HR08	4.30	0.45	2.924 ± 0.160	8.152 ± 0.359	7.82 ± 3.11	<10	<10
HR09	3.57	0.06	6.509 ± 0.288	7.784 ± 0.089	5.01 ± 0.65	**$1.4 \times 10^{6***}$**	<10
HR10	3.81	0.42	5.352 ± 0.370	8.467 ± 0.027	6.69 ± 0.33	1.09×10^5	<10
HR11	4.36	0.61	4.777 ± 0.121	4.586 ± 3.681	8.47 ± 2.01	4.2×10^3	<10
HR12	4.30	0.43	6.336 ± 0.360	8.091 ± 0.612	6.27 ± 0.80	<10	<10
HR13	4.60	0.67	3.643 ± 0.132	11.266 ± 0.072	8.66 ± 3.85	<10	<10
HR14	4.32	0.91	5.696 ± 0.235	9.888 ± 0.343	6.79 ± 4.00	<10	<10
HR15	3.79	0.62	4.561 ± 0.763	7.248 ± 0.331	7.83 ± 3.18	2.9×10^4	2.5×10^3
HR16	3.87	0.18	2.670 ± 0.070	9.345 ± 0.134	5.93 ± 2.09	1.0×10^3	<10
HR17	6.03	0.67	7.925 ± 1.860	11.802 ± 1.215	9.90 ± 0.51	**$1.5 \times 10^{6***}$**	**$7.6 \times 10^{5***}$**
HR18	4.44	0.73	6.918 ± 0.389	10.077 ± 0.142	8.05 ± 0.51	6.6×10^1	1.4×10^1
HR19	4.94	1.20	3.464 ± 0.142	10.872 ± 0.648	8.45 ± 3.30	3.8×10^4	5.3×10^2
Mean ± SD of 19 samples	4.374 ± 0.562	0.585 ± 0.273	4.714 ± 1.572	8.666 ± 1.827	7.54±1.32	-	-
Criteria	≤10%	≤2%	-	-	≤10%	$\leq 5.0 \times 10^5$	$\leq 5.0 \times 10^3$

***These results were not passed criteria of the Thai herbal pharmacopoeia requirements

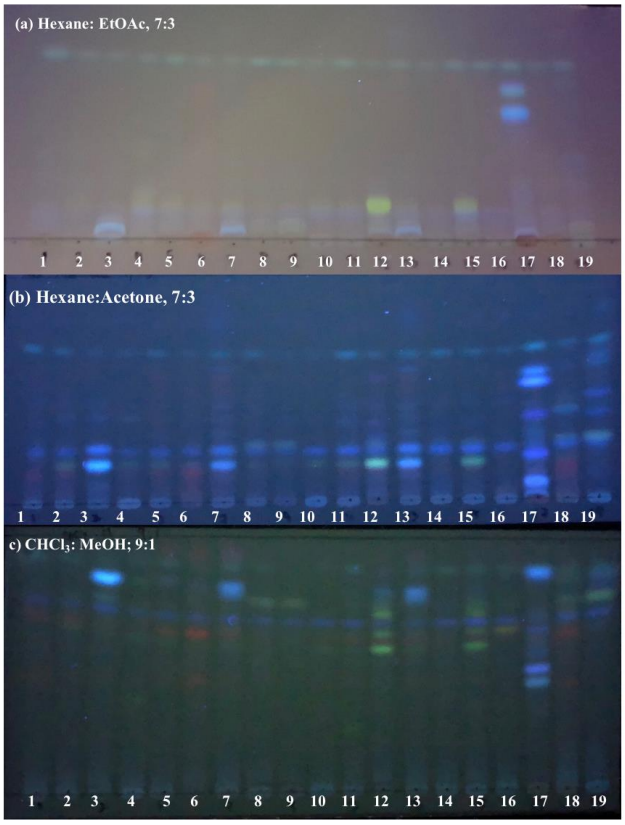

Figure 1 TLC fingerprint of HR01-19 in different 3 solvent systems (a) Hexane: EtOAc; 7:3 (b) Hexane: Acetone; 7:3 and (c) $CHCl_3$: MeOH; 9:1

HPLC fingerprint for similarity analysis of samples

Similarity of HPLC fingerprints were analyzed by using HCA and PCA. Peak area at retention time between 10.0 min to 30.0 min which almost found in chromatogram of all Harak samples were selected as the common peaks subjecting to HCA and PCA. Each sample was analyzed for three replicates to ensure that same sample can be grouped in same group by using the selected method. For HCA, average linkage was selected as between group linkage method. Manhattan distance method was selected to establish cluster due to it can distinguish HR17 which known as negative control from other clusters. Heatmap and HCA (Figure 4) clustered samples into four groups. Cluster A (12 samples) was the most related to HR19 consisting of HR01, HR02, HR03, HR04, HR05, HR07, HR10, HR11, HR13, HR14, HR15 and HR16. Cluster D composes of only one sample, HR17, which was the most different sample distinguished from the authentic Harak. PCA separated sample into 5 groups shown in Figure 5. Group A (HR01, HR02, HR03, HR04, HR05, HR07, HR10, HR11, HR13, HR14, HR15, HR16) consisted of 12 samples as same as the results of HCA. HR18 was the most correlated to authentic Harak HR19. Group B (HR06) and D (HR09) were also following HCA while group C was HR08 and HR12. Lastly, group E (HR17) was the most different from HR19 and other groups.

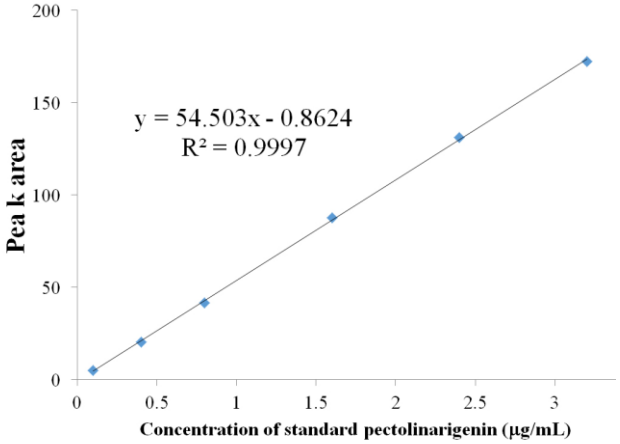

Figure 2 Calibration curve of standard pectolinarigenin

Table 5 Pectolinarigenin content in different samples of Harak powder and % difference between authentic and samples.

Samples	Pectolinarigenin content (mg/g drug powder) (Mean ± SD)	% Difference between authentic and samples
HR01	2.4±0.09	-90.5
HR02	22.1±0.09	-12.6
HR03	14.6±0.38	-42.2
HR04	19.7±0.88	-22.4
HR05	12.9±0.40	-49.1
HR06	30.0±0.53	18.5
HR07	16.4±0.66	-35.4
HR08	24.5±0.20	-3.2
HR09	18.1±0.09	-28.4
HR10	24.9±0.69	-1.5
HR11	11.8±0.07	-53.6
HR12	21.4±0.41	-15.3
HR13	15.3±1.09	-39.4
HR14	70.8±2.15	179.7
HR15	ND*	NT**
HR16	27.3±0.73	7.8
HR17	ND*	NT**
HR18	18.3±0.63	-27.9
HR19 (Authentic)	25.3±0.31	0

*ND = cannot be detected
**NT = No Test

Discussion

There are some reports of standard specifications of Thai herbal medicine remedy such as Hom-na-wa-khot, Hom-in-ta-juck, Chan-ta-lee-la and Leung-pid-sa-mud [21]. Thai Herbal Pharmacopoeia (THP) is a standard method for inspected standard specifications of herbal medicine in Thailand which is used to study standard specifications in many researches.

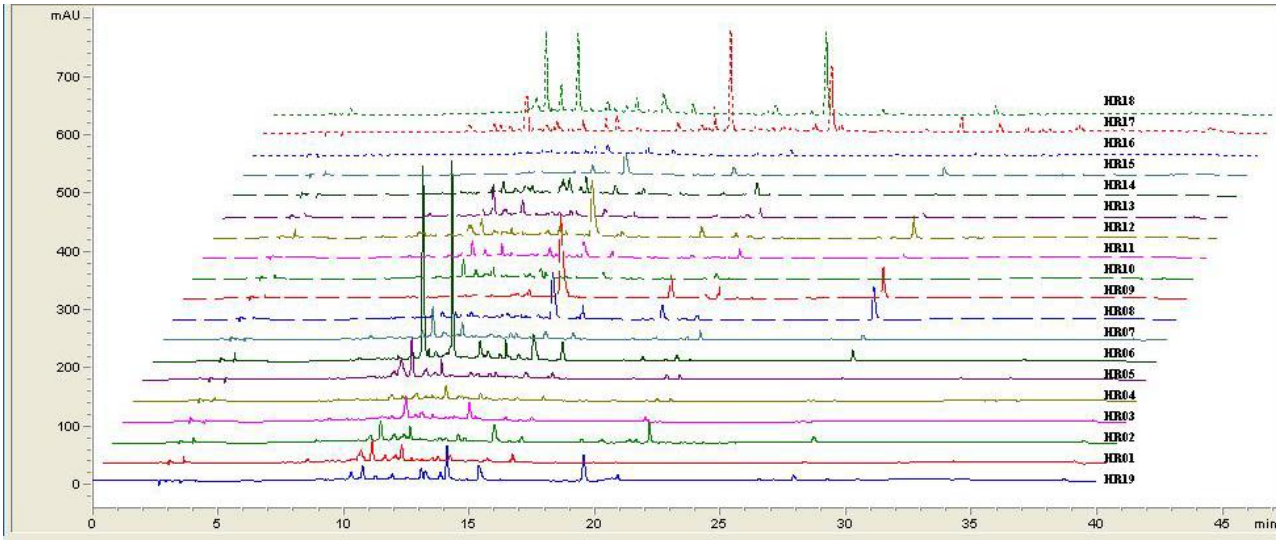

Figure 3: The HPLC chromatogram of the authentic specimen and the sample specimens; *** Retention Time (RT) of Pectolinarigenin=20.9 min.

Eighteen samples were collected from 14 provinces of Thailand, most of powder were a light brown which contained in colorless capsules no.1 as same as authentic. Only HR17 powder was red so it might be adulterated by another plant. The individual plant of Harak remedy has previously reported the morphological and histological characters (macroscopic and microscopic) of their roots [25]. Identification of crude drugs and capsule drugs based on morphological, anatomical and chemical fingerprint using TLC showed all commercial crude drugs and product capsules contained stem adulteration [5]. All samples passed the criteria of %total ash, %acid insoluble ash, %loss on drying. The mean of water soluble extractive value was 8.67% which was two times of the ethanol soluble (4.71%). Obviously, HR17 showed highest percentage of total ash, ethanol soluble and water soluble extractive value, and loss on drying which were 6.03, 7.93, 11.80, 9.90, respectively. Total aerobic microbial of HR09 and HR17 were 1.4 $x10^6$ and 1.5 $x10^6$ CFU/g which is higher than standard criteria (≤5.0 $x10^5$ CFU/g). Moreover, HR17 and HR03 also contaminated by yeasts and molds which was 7.6 $x10^5$ and 8.5 $x10^3$ CFU/g while it should be less than 5.0 $x10^3$ CFU/g. The results of standard specifications found that 15 samples were standard medicines (83.33%), three samples (16.66%) were non-standard because of the contamination.

The chemical constituents of aqueous extract and ethanolic extract of authentic by GC-MS were totally different. The ethanolic extract found that top three were oleic acid (32%), 17-Octadecen-14-yn-1-ol (23.99%) and Linoleic acid (11.12%). There are 25 compounds of ethanolic extract which were less than 1%. The highest percentage of aqueous extract was 2-naphthalenol (15.17%) followed by cyclooctasiloxane or hexadecamethyl (10.48%) whereas the lowest was lucenin 1.44%. The chemical fingerprints of 19 samples were investigated by TLC and HPLC. It is reasonable to have a difference color, TLC fingerprints of HR17 revealed the divergent bands when compare with authentic and others. Pectolinarigenin was used to evaluate the quality control by HPLC technique, authentic were 25.3±0.31 mg/g drug powder. HR16, HR10, and HR08 showed the similar content of pectolinarigenin to authentic (% differences less than 10). Following the previous report, pectolinarigein was 18.50 mg/g of extract or 0.18% w/w [20]. The powders definitely have lower concentrate constituents than the extracts and some samples might have small amount of pectolinarigenin. So, it possible that HR15 and HR17 were not detected. However, it is necessary to finding the other active compound which is more abundant for being a good marker. Additionally, the correlation of each sample under the HPLC peaks was examined by HCA and PCA. Twelve samples (66.67%) were the most related to authentic. HR17 was certainly different contamination, TLC bands and HPLC fingerprints which were effect on HCA and PCA, respectively. From these results, the pectolinarigenin should not be appropriate for a marker compound of this remedy because it was found in a small amount, whereas some other peaks represented a higher quantity. Thus, the dominant peak which is interesting should be further studies on compound isolation and biological activity for finding a marker compound before development of quality control. Moreover, other major chemical compounds that can be found in HPLC chromatogram such as O-methylalloptaeroxylin should be investigate for their antipyretic related activities.

Conclusion

Harak remedy consists of five root plants, so it might be adulterated by the others. Although there have only 44.44% were registered, 83.33% of them were standard. The contamination by microorganism is the main cause of non-standard medicine (16.66%).

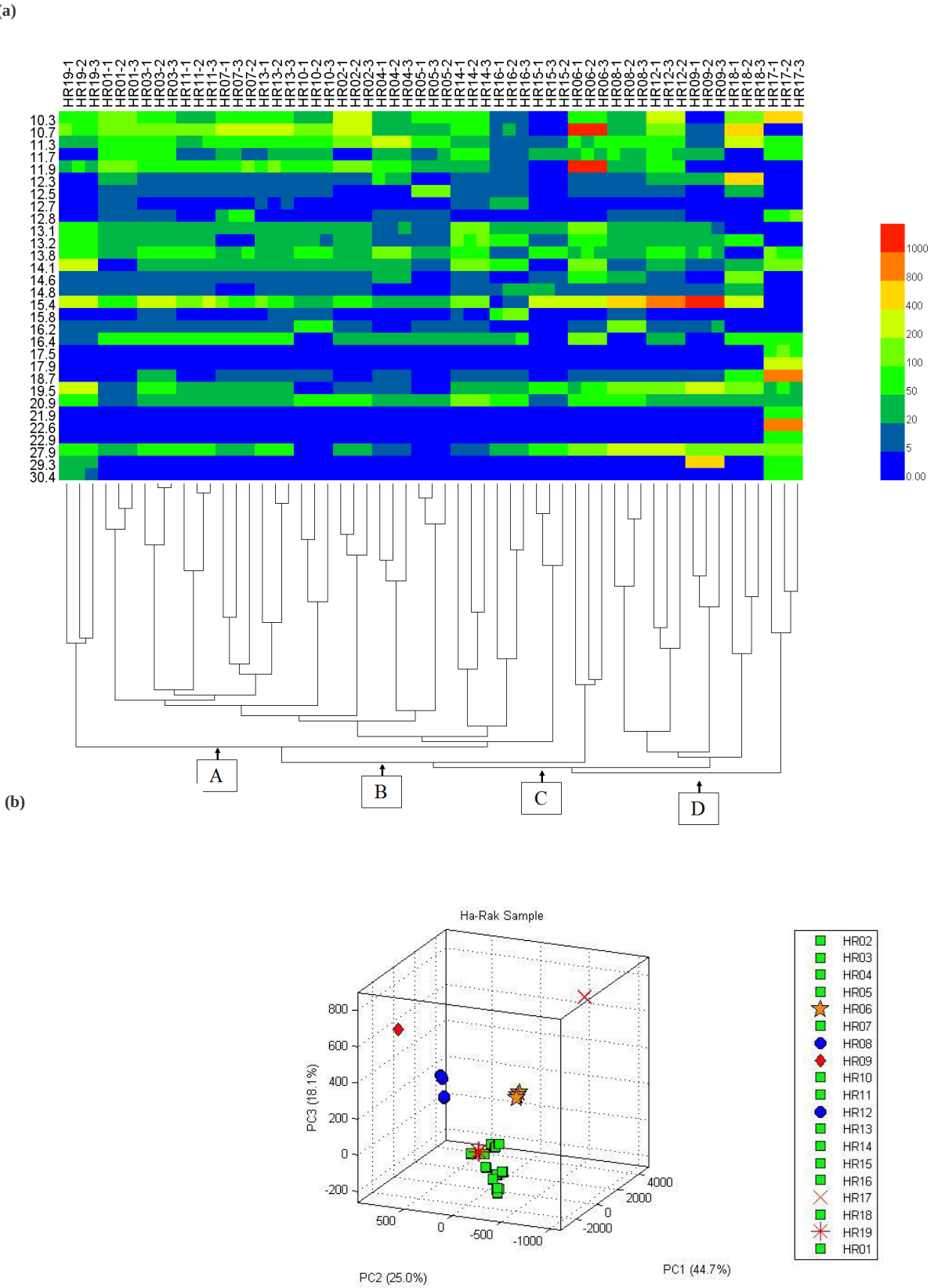

Figure 4 (a) Hierarchical clustering analysis (HCA) of Harak powder samples. Samples HR01-HR19 are performed using HemI statistics software, Heatmap Illustrator, Version 1.0; (b) Principal component analysis (PCA) of Harak powder samples. Samples HR01-HR19 are evaluated using MATLAB software

Discriminating color of drug powder is the first things to identify the others plant adulteration. However, there are many plants which have the same colors. So, the chemical fingerprint is also necessary to determine their components. Analytical techniques including TLC, HPLC, HCA and PCA showed the similar results. The most of samples were correlate to authentic whereas one of them was certainly different. The pectolinarigenin can be a chemical marker in *Harrisonia perforata*, but not appropriate to the formulary. Its content in the formulary does not represent the identity and the purity of the formulary. Nevertheless, this study gives the preliminary information for standardization and quality control of Harak remedy.

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