Chromatographic Fingerprinting and Physicochemical Characteristics of Kaff Maryam (*Anastatica hierochuntica* L.)

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Abstract

Kaff Maryam (*Anastatica hierochuntica* L.) is a desert plant native to Arab countries where it has long been used as a traditional medicine for the treatment of menstrual ailments. Despite its medicinal values, the availability of the information required for quality control of this medicinal plant is still inadequate. Therefore, the investigation on the TLC fingerprinting and physicochemical characteristics of Kaff Maryam was carried out. The plant materials were procured from localities in Egypt, Saudi Arabia, and Jordan. The physicochemical studies were conducted based on WHO guidelines. From the results, sand and plastic debris, accounting for 0.16 ± 0.18% w/w, were the main foreign matters found from the raw materials. The moisture content of the powdered samples was 5.29 ± 0.17% w/w, which did not exceed the standard limit of 10%. From the ash study, the contents of total ash and acid-insoluble ash were 6.96 ± 0.15 and 0.45 ± 0.13% w/w, respectively. The water extract quantity (1.90 ± 0.18% w/w) was greater than that of the ethanol extract (0.83 ± 0.02% w/w), while the colorless volatile oil was obtained with a minute amount (0.67 ± 0.40% v/w) upon hydrodistillation. The TLC fingerprinting of the methanol extracts using taxifolin as a chemical marker led to the detection of 16 spots under ultraviolet lights as well as after visualization with *p*-anisaldehyde which the hR_f of 51 indicated the presence of taxifolin. The generated information from this study would be useful in the further development of a suitable specification of Kaff Maryam.

Keywords: *Kaff Maryam, Anastatica hierochuntica, Chromatographic fingerprinting, Physicochemical characteristics*

1. Introduction

Anastatica hierochuntica L., commonly called Kaff Maryam, is a small annual plant native to Arab countries and thrives in the Sahara-Arabian deserts (Zin, Kassim, Alshawsh, Hashim, & Mohamed, 2017). It was prescribed in Egyptian folk medicine and considered as bringing good luck for childbirth (Mohamed, Khalil, & El-Beltagi, 2010), while nowadays it is medicinally used to facilitate labor and to treat menstrual disorders such as dysmenorrhea, emmenagogue, and miscarriage (AlGamdi, Mullen, & Crozier, 2011). These health benefits of Kaff Maryam would be due to the estrogenic effects of its phytoconstituents which was previously found to reduce the side effect of estrogen hormone and to stimulate luteinizing hormone production in female mice (Baker, Mohammd, Ali, & Jameel, 2013). Furthermore, it is given to cure gastrointestinal tract diseases, hypertension, and infertility (AlGamdi et al., 2011). Kaff Maryam is a plant attractive not only to people in Arab countries, but also to Asian people in Malaysia, Indonesia, and southern provinces of Thailand who use this plant to promote labor and to ease menstrual pain (Affandi, 2018; Kim & Lean, 2013). Additionally, the herbs were consumed as an infusion of the whole plant in hot water or as a powder in a capsule.

For nutritional components, Kaff Maryam is rich in minerals including Ca, Co, Cr, Cu, Fe, Mg, Mn, and Zn. Its phytoconstituents comprise phenolics, flavonoids, lycopene, and β-carotene (Daur, 2012; Mohamed et al., 2010). Its crude extracts displayed a wide range of biological activities including antioxidation, antimicrobial, antidyslipidemic, anti-inflammatory, liver protective, and antimelanogenesis activities (Zin et al., 2017). Several separation techniques such as high-performance liquid chromatography

(HPLC) with photodiode array (PDA) and tandem mass spectrometric (MS^2) detection (AlGamdi et al., 2011), gas chromatography-mass spectrometry (GC-MS) (Saranya, Ali, & Anuradha, 2019), and conventional column chromatography, as well as structural elucidation techniques (Yoshikawa, Xu, Morikawa, Ninomiya, & Matsuda, 2003) were developed to separate and identify chemical constituents from Kaff Maryam. To date, bioactive components that exhibited biological activities corresponding to those effects found in the crude extracts have been isolated. They were phenolic compounds mainly belonging to the flavonoid class such as the hepatoprotective anastatins A and B, the antioxidant taxifolin, and the antimelanogenesis isosilybins A and B (AlGamdi et al., 2011; Nakashima et al., 2010; Yoshikawa et al., 2003). Among these, taxifolin, also known as dihydroquercetin, was shown to be a predominant constituent in the whole plant methanolic extract of Kaff Maryam (Yoshikawa et al., 2003), suggesting that this main compound could be used as a chemical marker for quality control of Kaff Maryam.

Based on the SciFinder Scholar database, there has been no report on thin-layer chromatography (TLC) fingerprints and physicochemical properties of Kaff Maryam so far. This together with the interesting bioactivities and that family members of the authors usually use this herbal plant, we were thus interested in the study on TLC fingerprints and physicochemical characteristics of Kaff Maryam. In the present work, TLC profiling and physicochemical analysis were carried out with Kaff Maryam collected from 3 different localities including Egypt, Saudi Arabia, and Jordan. The collection of the plants was mainly based on the easy availability and reliability of them in the central market of each country. The obtained data on its TLC fingerprints and physicochemical properties would give rise to the further development of a suitable specification of raw materials from Kaff Maryam.

2. Objectives

This research was aimed to investigate the TLC fingerprints and physicochemical characteristics of Kaff Maryam.

3. Materials and Methods

3.1 Chemical and materials

Analytical grade solvents including ethanol, ethyl acetate, and methanol were purchased from RCI Labscan, Thailand, while hydrochloric acid (37%), sulphuric acid (95-97%), *p*-anisaldehyde (98%), and toluene were provided from Merck, Germany. Formic acid (90%) was obtained from Ajax Finechem, Australia. Taxifolin (an analytical standard with 93.5% purity) used for TLC fingerprinting was purchased from Sigma-Aldrich, USA, and ashless filter papers (no. 42) were bought from Whatman, UK. TLC was performed on silica gel 60 F_{254} (Merck, Germany). Linomat 5 TLC sampler together with TLC visualizer (CAMAG, Switzerland), 100-mL microsyringe (Hamilton, Switzerland), and twin trough TLC chamber (20 x 10 cm) (CAMAG, Switzerland) were employed in this study.

3.2 Plant materials

The dried whole plants of Kaff Maryam were bought from 3 different localities in Arab countries including Cairo (Egypt), Mecca (Saudi Arabia), and Amman (Jordan). The plant was identified by Mr. Muhamanurudin Mudeng, a folk medicine doctor in Mueang Yala District of Yala Province in southern Thailand. The raw materials, except for those used for the foreign matter study, were firstly cleaned with water and then dried in a hot air oven at 50 °C for 8 hr. After that, the dried samples were ground into a fine powder and sieved over a test sieve no. 60 (250 μm). The fine powder was contained in a zip bag and kept in a desiccator until further use.

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3.3 TLC fingerprinting

Taxifolin, a major constituent of Kaff Maryam (Yoshikawa et al., 2003), was used as a chemical marker for TLC fingerprinting of the extracts. Its chemical structure was also illustrated in Figure 1.

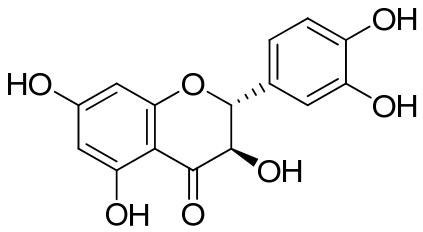

Figure 1 The chemical structure of taxifolin

Each powder sample was extracted with methanol overnight and then evaporated to dryness. After that, a sample solution with a concentration of 10.0 mg/mL was prepared in methanol, while that of taxifolin was prepared in a concentration of 2.0 mg/mL. The sample solutions were spotted on 20 x 10 cm TLC plates as a band of 6.0 mm with a constant application rate of 150 nL/s, and a space distance between two bands was 8.9 mm. The development of the plate was carried out in a TLC chamber pre-saturated with 20 mL of a suitable mobile phase at room temperature for 20 min prior to use. The distance of the chromatogram run was 80 mm. After complete development, the plate was removed and left to dry in a fume hood for 10 min. The developed plate was photographed under UV254 and UV366 as well as after staining with a *p*-anisaldehyde-sulfuric acid solution and subsequent heating. After the spots were detected, the center of the spot for each component was marked, and the distance travelled by each component was measured. Their hR_f values were then calculated by using the following equation. The identity of taxifolin present in the extracts was confirmed on the basis of the hR_f value and general appearance of the spot under the abovementioned detection compared to those of the standard taxifolin.

$$hR_f = \frac{\text{distance travelled by the component}}{\text{distance travelled by the solvent}} \times 100$$

3.4 Physicochemical studies

Physicochemical parameters (foreign matter, moisture content, total ash content, acid-insoluble ash content, extractive value, and volatile oil content) were investigated based on WHO guidelines (World Health Organization, 2011). Each sample was studied in triplicate, and the methods were carried out as follows:

3.4.1 Foreign matter

100 g of each sample were examined for foreign matter by naked eyes and with the help of a magnifying glass. The collected foreign matter was weighed, and the amount was reported as a percentage.

3.4.2 Moisture content

Moisture content was studied by a loss-on drying method. 2 g of each sample were contained in a moisture can and then kept in a hot air oven at 105 °C for 4 hr. After cooling down the sample in a desiccator, the weight was measured. The final weight loss was calculated, and it represented the moisture content of the sample.

3.4.3 Total ash

2 g of each sample were contained in a crucible and then ignited in a muffle furnace at 500 °C for 5 hr to give white carbonless ash. The amount of total ash was expressed as a percentage of the original weight of the sample.

3.4.4 Acid-insoluble ash

To a crucible of the total ash sample, 25 mL of 10% hydrochloric acid were added. The crucible was covered with a watch glass and subsequently heated over a hot plate. The watch glass was rinsed with hot water, and the solution was contained in the same crucible. The mixture was filtered through an ashless filter

paper. The filter paper was washed with an appropriate volume of hot water until a pH of the obtained filtrate is neutral. The filter paper was then contained in the sample crucible, heated to dryness over a hot plate, and ignited in a furnace using the same condition as conducted in the total ash study. The amount of the ash was expressed as a percentage of the initial sample weight.

3.4.5 Extractive value

4 g of each sample were separately extracted with 100 mL of 95% ethanol and water in a conical flask with a stopper. The flask was shaken using an automatic shaker at 150 rpm for 6 hr and then kept overnight for another 18 hr. The mixture was filtered using vacuum filtration, and the volume of the corresponding filtrate was adjusted to 100 mL with the extracting solvent. After that, 25 mL of the filtrate were pipetted into an evaporating dish followed by evaporating to dryness over a water bath. Then, the evaporating dish was placed in a hot air oven at 105 ^{o}C for 6 hr. After cooling down the sample in a desiccator, the weight was measured and calculated in a percentage.

3.4.6 Volatile oil content

A Clevenger-type apparatus was used to extract the essential oils from the samples. Each sample (50 g) was placed in a distillation flask and subjected to hydrodistillation for 4 hr. After cooling down, the volume of vaporized oils collected in a graduated tube was read and expressed as a percentage of the original weight of the sample.

3.5 Statistical analysis

Data were expressed as mean ± SD of three determinations calculated by the Microsoft Excel program. Statistical analysis was performed by a one-way analysis of variance (ANOVA) followed by the Duncan′s test. Differences were regarded as significant at the level of $p < 0.01$.

4. Results and Discussion

The physical appearance of Kaff Maryam obtained from Cairo in Egypt, Mecca in Saudi Arabia, and Amman in Jordan was shown in Figure 2. The obtained dried whole plants appeared in a globose form which the branches and leaves curled inward to form a tight woody ball; the size was from 6 to 14 cm across. These appearances represented the characteristics of Kaff Maryam described in the medicinal flora of North Africa (International Union for Conservation of Nature, 2005). When the plant dries out, a reduction of the pressure in the cells causes the branches and leaves to curl, thus forming a seed reservoir. This mechanism protects the seeds and prevents their dispersal (Hegazy, Barakat, & Kabiel, 2006). Every single whole plant of Kaff Maryam from Cairo and Amman (Figures 2a and 2c, respectively) were brown in color, and their woody balls were bigger than that from Mecca (Figure 2b), the color of which was greenish-brown. These morphological data indicated that the two formers would be older than the latter.

a **b** **c**

Figure 2 The morphologies of Kaff Maryam from 3 different localities (a: Cairo in Egypt, b: Mecca in Saudi Arabia, and c: Amman in Jordan)

4.1 TLC fingerprint

In the TLC fingerprinting of the crude extracts, taxifolin, a major constituent of Kaff Maryam (Yoshikawa et al., 2003), was used as a chemical marker. The standard taxifolin solution (5 µL) and the sample solutions (each 4 µL) were applied on the same TLC plate. The optimized mobile phase for TLC development was obtained after running a TLC plate in different mobile phase compositions, during which a TLC co-spot method was performed as a preliminary step to confirm the identity of taxifolin present in the extracts. Besides, this technique can be useful in case a mobile phase runs with a slight diagonal. In the co-spotting lane, the TLC chromatogram displayed only one spot of taxifolin without an elongated shape in varieties of the tried mobile phase systems (data not shown). The best result was obtained by the use of a mixture of toluene/methanol/ethyl acetate/formic acid in a ratio of 56:4:30:10 (v/v). TLC chromatograms of the methanol extracts from 3 Kaff Maryam samples and the standard taxifolin were presented in Figure 3.

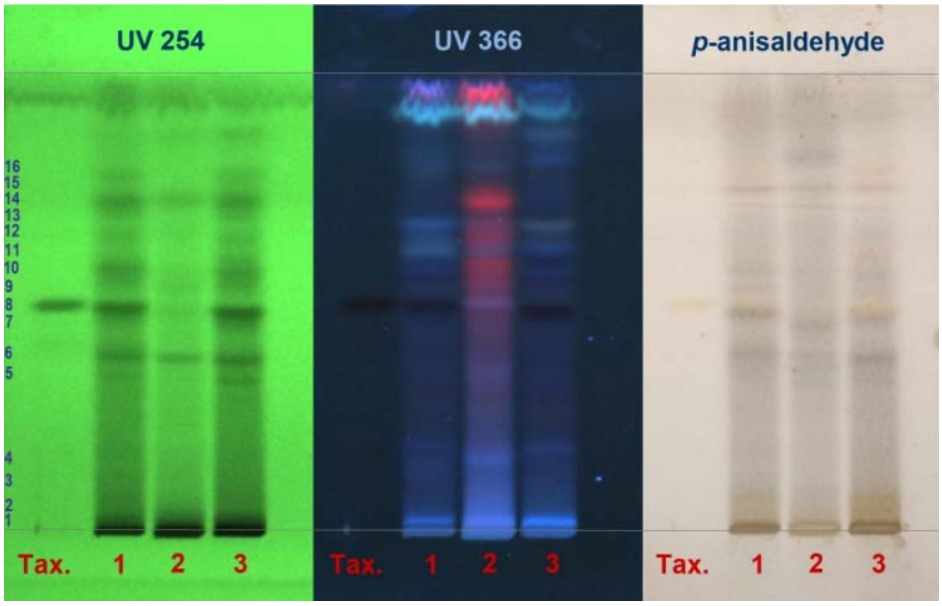

Figure 3 The TLC chromatograms of the standard taxifolin (Tax.) and the methanol extracts from Kaff Maryam (1: Cairo in Egypt, 2: Mecca in Saudi Arabia, and 3: Amman in Jordan)

Upon detection under UV254, UV366 and after visualization with a *p*-anisaldehyde-sulfuric acid solution, the TLC chromatograms of the methanol extracts from the samples 1 (Cairo in Egypt) and 3 (Amman in Jordan) closely resembled each other. 16 different spots were observed from the sample 1 with hR_f values of 16, 19, 23, 28, 39, 43, 50, 51, 54, 57, 60, 62, 65, 68, 70, and 73 (Table 1). With a comparison to the standard taxifolin, all the samples contained taxifolin which was positive at the spot no. 8 with the hR_f value of 51 and appeared as a light orange spot after staining with a *p*-anisaldehyde-sulfuric acid solution. In the sample 2, the weak quenching under UV254 of taxifolin was observed. This indicated the low amount of taxifolin present in the methanol extract of Kaff Maryam collected from Mecca in Saudi Arabia. This would be due to environmental conditions that affect the plant growth which could cause the variation in the quantity of taxifolin. In terms of physiological age, it was previously demonstrated that English ivy (*Hedera helix* L.) leaves in a mature phase accumulated greater levels of quercetin than those in a juvenile one (Murray & Hackett, 1991). This was in agreement with the previous study on the influence of plant age on phenylethanoid and phenylpropanoid contents of roseroot (*Rhodiola rosea* L.) which exhibited that significant increases in the two contents occurred with grown roseroot (Kołodziej & Sugier, 2013). For Kaff Maryam, knowledge of such influential factors is insufficient, and further investigation under controlled conditions should be carried out. From the TLC chromatograms under UV366, various fluorescent spots were

detected, indicating the presence of steroids, phenols, terpenes or essential oils components (Fried & Sherma, 1999).

Based on the SciFinder Scholar database, several chromatographic and spectroscopic techniques have been developed to isolate and identify chemical constituents from Kaff Maryam. AlGamdi et al. (2011) performed the isolation of various compounds from the aqueous seed extract of Kaff Maryam by HPLC-PDA-MS^2. The investigation indicated the presence of flavones as the most abundant compounds, followed by phenolic acids and hydroxycinnamates, respectively. The GC-MS analyses of hexane, ethyl acetate, and methanol extracts of Kaff Mayam's stem, seed, and leaf crude extracts by Saranya et al. (2019) led to the identification of various bioactive compounds including phenols, glucosinate, terpenes, esters, thiols, fatty acid amines, fatty acid alcohol, sterols, and hydrocarbons. The methanolic extract from the whole plants of Kaff Maryam upon silica gel column chromatography and HPLC yielded 36 compounds, among which taxifolin was the most abundant component constituted 0.044% of the isolated yield (Nakashima et al., 2010). To our knowledge, this is the first report on the fingerprinting of Kaff Maryam using a TLC technique, which is simple and cost-friendly compared to other chromatographic techniques. The developed approach could provide a complementary fingerprint material which might prove a sufficient choice for the more expensive instrumental fingerprinting. Nevertheless, multiple chromatographic fingerprints that enable a more comprehensive data analysis of Kaff Maryam should be further investigated.

Table 1 The hR_f values of chemical constituents in the methanol extract of Kaff Maryam from Cairo in Egypt

Spot no.	hR_f	Detection		
		UV254	UV366	*p*-anisaldehyde
1	16	-	Blue	-
2	19	-	-	Brown
3	23	-	Blue	-
4	28	-	Blue	-
5	39	Weak quenching	-	-
6	43	Quenching	-	Gray
7	50	-	-	Gray
8*	51	Quenching	Dark blue	Light orange
9	54	-	-	Gray
10	57	Weak quenching	Blue	Light blue
11	60	-	Light blue	-
12	62	Weak quenching	-	-
13	65	-	Light blue	-
14	68	Weak quenching	-	-
15	70	-	-	Purple
16	73	Weak quenching	-	-

*taxifolin

4.2 Physicochemical properties

In this study, the samples from each locality were investigated in triplicate, and the results were summarized in Table 2. From 3 Kaff Maryam sources, it showed that sand and plastic debris, ranging between 0.04% and 0.38%, were the main foreign matters found from the raw materials. This was due to their habitats in deserts, while the plastic remains would come from a plastic container used in the storage and transportation of the plant materials. For the moisture content of Karr Maryam, it was found that the contents (4.92-5.70%) did not exceed the standard limit of 10% (World Health Organization, 2011). Apart from water which is a component found in plant cells, the moisture found in Kaff Maryam would be a result of the absorption of moisture from the air by polar glycosides (Ghazanfar, 1994) and sugar components (Zin et al., 2017), both contents of which constituted 40.48-42.68% in stems, seeds, and leaves of Kaff Maryam (Saranya, Ali, Anuradha, & Safia, 2019). Concerning the effect of plant age, it demonstrated that the total ash contents of Kaff Mayam were significantly varied (4.75-9.37%). Additionally, the younger plant (Kaff Maryam from Mecca) could uptake more inorganic minerals than the other older twos (Kaff Maryam from Cairo and Amman), thereby resulting in the greater total ash content of 9.37% (Jungk & Barber, 1975). Among them,

however, Kaff Maryam from Mecca gave the lowest quantity of acid-insoluble ash (0.14%), owing to the low amount of sand residue found on the smaller plant.

Table 2 The physicochemical characteristics of Kaff Maryam from 3 different localities

Sources	Foreign matter	Moisture	Total ash	Acid-insoluble ash	Ethanol extractive	Water extractive	Volatile oil
Cairo	0.07 ± 0.06	4.92 ± 0.14[a]	6.77 ± 0.05[a]	0.92 ± 0.20[a]	0.69 ± 0.02[a]	0.78 ± 0.05[a]	0.67 ± 0.23
Mecca	0.04 ± 0.01	5.25 ± 0.05[b]	9.37 ± 0.13[b]	0.14 ± 0.08[b]	0.98 ± 0.02[b]	3.34 ± 0.30[b]	0.67 ± 0.61
Amman	0.38 ± 0.31	5.70 ± 0.09[c]	4.75 ± 0.22[c]	0.30 ± 0.09[b]	0.82 ± 0.02[c]	1.57 ± 0.04[c]	0.67 ± 0.23
average	0.16 ± 0.18	5.29 ± 0.17	6.96 ± 0.15	0.45 ± 0.13	0.83 ± 0.02	1.90 ± 0.18	0.67 ± 0.40

Values were represented in % w/w except for those of volatile oil content (% v/w). The same superscript letter showed no significant difference between sources ($p < 0.01$). Averages were shown as grand mean ± pooled SD.

Upon maceration with 95% ethanol and water, Kaff Maryam yielded higher amounts of the water extracts (0.78-3.34%) than those of the ethanolic ones (0.69-0.98%). This indicated that water dissolved polar carbohydrate, polyphenolic and glycoside contents in Kaff Maryam better than 95% ethanol. For volatile oil content, the colorless oils were obtained in a low amount with an average percentage of 0.67%. This result was in agreement with the previous investigation which only 0.05% of the oil was obtained from 250 g of the dried aerial parts (Abd El-Gaber, El Gendy, Elkhateeb, Saleh, & El-Seedi, 2018). Upon GC-MS analysis, they found that the first four main components of the oil were cuminaldehyde (34.82%), *trans*-β-caryophyllene (25.17%), L-linalool (7.82%), and (–)-caryophyllene oxide (3.56%), respectively.

From the statistical analysis of the obtained data, it exhibited that no statistical difference between 3 plant sources was observed only in the data of foreign matter and volatile oil. Additionally, the data of the other characteristics displayed significant differences between the sources except for the acid-insoluble ash of which the data obtained from Mecca and Amman were not significantly different from each other. These results revealed the variation in the data obtained from different plant sources which could be due to geographical differences as the plants were procured from 3 different countries. Thus, from this study, it is worth noting that vital factors such as plant age, places of collection, and proper numbers of sample sources play an important role in the further establishment of a precise specification of Kaff Maryam.

5. Conclusion

As there has been no enough evidence for the TLC fingerprinting and physicochemical investigation on the whole plant of Kaff Maryam (*Anastatica hierochuntica* L.), the present work was carried out. For the TLC fingerprints, taxifolin was shown to be a major component of Kaff Maryam, excluding that from Mecca in Saudi Arabia. The generated information on physicochemical characteristics would be useful in the further development of a suitable specification of Kaff Maryam. Nevertheless, to ensure the precision of the data to be used for the construction of the aforementioned standard, further investigation of Kaff Maryam should be paid attention to environmental factors, plant age, places of collection, and proper numbers of sample sources. Also, the study on other information such as macro- and microscopical data, microbial contamination and heavy metal contamination of the raw materials should be conducted.

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