

CHEMICAL COMPOSITION AND ANTIOXIDANT ACTIVITY OF *HEDYCHIUM MALAYANUM* ESSENTIAL OILS

(Komposisi Kimia dan Aktiviti Anti-agen-pengoksidaan Minyak Pati *Hedychium malayanum*)

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Abstract

The leaves and stems of *Hedychium malayanum* were hydrodistilled using a Clevenger-type apparatus to give 0.038 and 0.009% oils, respectively. Sixteen and seven compounds which made up 98.4 and 100% of the respective oils were identified using capillary GC and GC-MS with a DB-5 column. These oils contained high amount of monoterpene hydrocarbons with β -pinene being the main compound found in the leaves (39.1%) and the stems (46.7%) followed by α -pinene (22.3%) and β -caryophyllene (13.7%) which was found in the leaf oil and 1,8-cineole (17.7%) and α -pinene (16.9%) in the stem oil. Antioxidant test by 1,1-diphenyl-2-picrylhydrazyl (DPPH) showed that the leaf oil was a stronger antioxidant (31.1%) than Trolox (28.4%), whereas the stem oil was the weakest antioxidant (23.9%). β -Carotene bleaching (BCB) in linoleic acid showed that both oils were weaker antioxidants than butylated hydroxytoluene (96.8% inhibition). However, the stem oil (79.8%) showed a good inhibition whereas the leaf oil possessed a moderate capacity (54.7%) to prevent the oxidation by linoleic acid.

Keywords: *Hedychium malayanum*, essential oil, chemical composition, GC-MS, antioxidant activity

Abstrak

Daun dan batang *Hedychium malayanum* telah dihidrosuling menggunakan radas jenis-Clevenger bagi menghasilkan 0.038 dan 0.009% minyak, masing-masingnya. Enam-belas dan tujuh sebatian yang melibatkan 98.4 and 100% bagi masing-masing minyak telah dikenal pasti menggunakan KG rambut dan KG-SJ dengan turus DB-5. Minyak-minyak ini mengandungi amuan hidrokarbon monoterpena yang tinggi dengan β -pinena merupakan sebatian utama yang ditemui dalam daun (39.1%) dan batang (46.7%) diikuti dengan α -pinena (22.3%) dan β -kariofilena (13.7%) yang ditemui dalam minyak daun dan 1,8-sineol (17.7%) and α -pinena (16.9%) dalam minyak batang. Ujian anti-agen-pengoksidaan dengan 1,1-difenil-2-pikrilhidrazil (DPPH) menunjukkan bahawa minyak daun adalah anti-agen-pengoksidaan yang lebih kuat (31.1%) berbanding Trolox (28.4%), manakala minyak batang adalah anti-agen-pengoksidaan yang paling lemah (23.9%). Pelunturan β -karotena (PBK) dalam asid linoleik menunjukkan kedua-dua minyak adalah anti-agen-pengoksidaan yang lebih lemah daripada hidroksitoulena terbutil (96.8% perencatan). Walau bagaimanapun, minyak batang (79.8%) menunjukkan perencatan yang baik manakala minyak daun memperlihatkan kapasiti sederhana (54.7%) untuk menghalang pengoksidaan oleh asid linoleik.

Kata kunci: *Hedychium malayanum*, minyak pati, komposisi kimia, GC-MS, aktiviti anti-agen-pengoksidaan

Introduction

There are 151 Zingiberaceae species belonging to 18 genera that can be found in Peninsular Malaysia. *Alpinia* with 23 species is the most numerous genus under Zingiberaceae whereas *Hedychium* with 7 species ranks the eighth [1]. Recently, over 50 *Hedychium* species have been recognized from many distributions, mainly eastern Himalayas to south India, south China and south-east Asia [2]. The characteristic features of the genus *Hedychium* are the terminal inflorescence with 2-3 flowers to each bract, the long staminodes and stamen and deeply bilobed lip [3]. Holttum [3] described *Hedychium malayanum* by its leaves to reach to 35 cm in length with longest leaves 8-11 cm in width, glabrous and widest 1/4 -1/3 from the apex; its petiole to about 4 cm in length; its inflorescence from the

base of lowest bract to the tip of apical one is 14 cm in length; and its bracts are very broad, overlapping, glabrous and each bract enclosing 3 or more flowers. This species has been found to grow in two locations of Fraser's Hill and Cameron Highlands, and is to be expected at other places on the main range. In contrast to the wide use of *Hedychium coronarium* in the culinary field and traditional medicine, the uses of *Hedychium malayanum* have not been explored yet.

Essential oils were described as having preservative property to avoid lipid deterioration, oxidation and spoilage by microorganisms [4]. Because of the potential role of essential oils as a source of natural antioxidants and biologically active compounds, essential oils have attracted a great deal of scientific interest [5]. Essential oils rich in monoterpenes are recognized as food preservatives, and monoterpene essential oils are natural antioxidants that are active against certain cancers such as breast, liver, colon, and prostate cancers, and leukemia [6]. They are also considered to be important in diets or medical therapy for biological tissue deterioration due to free radicals [7].

To our knowledge, no study has been done on the chemical constituents and antioxidant activity of *Hedychium malayanum* essential oils. In this study, we identify the chemical components and evaluate the antioxidant activity of the leaf and stem oils of *H. malayanum*. Several studies have been conducted on the essential oils from other *Hedychium* species, such as Malagasy *H. coronarium* [8], *H. spicatum* [9], Brazilian *H. coronarium* [10], *H. gardenarium* [11], *H. acuminatum* [12], *H. larsenii* [13], *H. thyriforme* [14], *H. elatum* [14], *H. bousigoniamum* [14], *H. forrestii* [14], *H. coccineum* [14], *H. flavescens* [14], *H. stenopetalum* [15], Vietnamese *H. coronarium* [15], *H. flavum* [15], *H. ellipticum* [15] and French Polynesia *H. coronarium* [16].

Materials and Methods

Plant Materials

The leaves and stems of *Hedychium malayanum* were collected in December 2013 from Fraser Hills, Pahang, Malaysia and stored fresh in the freezer at -20°C . A voucher specimen of WAY 538 was deposited at the Universiti Kebangsaan Malaysia Herbarium.

Isolation Procedure

Each of the frozen fresh leaves and stems were cut into small pieces, blended in distilled water and hydrodistilled in a Clevenger-type apparatus for 3 hours. The essential oils obtained were dried over anhydrous sodium sulfate, transferred into small vials covered with aluminium foil, and kept in a freezer at -20°C . Each of the oils was dissolved in *n*-hexane prior to analysis using gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS).

Gas Chromatography

GC analyses were performed by using a DB5890 equipped with a flame ionization detector (FID). A fused silica capillary column of DB-5 (30 m X 0.32 mm id, film thickness 1.50 μm) was used. The operating conditions of this column were as follows: initial oven temperature, 50°C for 3 min, then raised at $3^{\circ}\text{C}/\text{min}$ to 200°C , and then held for 5 min; injector and detector temperatures, 250°C ; carrier gas, 1.0 mL/min He; injection volume, 0.2 μL . Peak areas were determined with a DB-chem-software.

Gas Chromatography-Mass Spectrometry

GC-MS analyses were carried out using an Agilent 7890A GC equipped with the mass spectrometer (Agilent 5975C inert MSD, with Triple-Axis Detector). A fused silica capillary column was used: DB-5 (30 m x 0.25 mm id, film thickness 0.25 μm). The operating conditions of this column were as follows: initial oven temperature, 50°C for 3 min, then to 200°C at $3^{\circ}\text{C}/\text{min}$ then held for 5 min; injector and detector temperatures, 250°C ; carrier gas, 1.2 mL/min He; split ratio, 10:1; injection volume, 1.0 μL . Operating parameters: ionization voltage, 70 eV; ion source temperature, 200°C ; scan mass range, 40-350 amu. Peak areas were determined with MSD ChemStation from Agilent Technologies. A library search was carried out for all peaks using the NIST Mass Spectral Library.

Essential Oil Components Identification

The essential oil constituents' identification was based on a comparison of their Kovats retention indices relative to C₈-C₂₀ *n*-alkanes, with those of the chemical components gathered by Adams [17], Choi [18] and Hognadottir and Rouseff [19]. Compounds were further identified using their MS data compared to the NIST mass spectral library.

Antioxidant Activity

DPPH Radical Scavenging Assay

Radical scavenging activity was determined by a spectrophotometric method based on the reduction of ethanol solution of DPPH using the method of Choi et al. [20]. Each of the oils (10 µL) was mixed with 900 µL of 100 mM Tris-HCl, 40 µL of ethanol, 50 µL of 5% (w/w) Tween 20 solution and then added to 1 mL of 0.5 mM DPPH in ethanol. The mixture was wrapped with aluminium foil, shaken with a mechanical shaker (Vortex) and left to stand for 30 min at room temperature. The control solution was prepared using 10 µL of distilled water instead of 10 µL of oil. Trolox, a standard antioxidant, was prepared using 50 µL of 1 mM Trolox-ethanol instead of 10 µL of oil and 40 µL of ethanol. For each solution, three replicates were recorded. The disappearance of DPPH was read spectrophotometrically at 517 nm using a spectrophotometer (Shimadzu UV-Mini-1240). The radical-scavenging activity of the oils and Trolox (standard), expressed as percentage inhibition of DPPH, was calculated according to the following formula equation 1:

$$\text{Inhibition percentage (\%)} = [(A_c - A_s)/A_c] \times 100 \quad (1)$$

where, A_c is the absorbance of the control mixture (DPPH-ethanol) excluding the test solutions (oils/Trolox), and A_s is the absorbance of the test solutions (oils/Trolox).

β -Carotene Bleaching (BCB) Test

The antioxidant activity of oils was evaluated by the spectrophotometric β -carotene bleaching test of Sacchetti et al. [4] with a modification. A stock solution of β -carotene-linoleic acid mixture was prepared as follows: 10 mg of β -carotene was dissolved in 10 mL of chloroform; this β -carotene-chloroform solution (0.2 mL) was pipetted into a boiling flask containing 20 mg of linoleic acid and 200 mg of Tween 40. Chloroform was completely evaporated by using a rotary evaporator at 40°C for 5 min. To the residue, 50 mL of distilled water was slowly added with vigorous shaking to form an emulsion. This emulsion (5 mL) was added to a tube containing 0.2 mL of the oil solution prepared by diluting 4 mg of the oil in ethanol to 2 mL and the absorbance was immediately measured at 470 nm ($t = 0$) against a blank consisting of an emulsion without β -carotene. The tubes were placed in a water bath at 50°C and the oxidation of the emulsion was monitored spectrophotometrically by measuring absorbance at 470 nm at every 30 min for 120 min. Control contained 0.2 mL of distilled water instead of the oils. Butylated hydroxytoluene (BHT), a stable antioxidant, was used as a synthetic reference. Its solution was prepared accordingly like the above oil solution whereby the oil was replaced with BHT (4 mg of this was dissolved in ethanol to 2 mL). For each solution, three replicates were recorded. The rate of β -carotene bleaching was calculated using the following equation 2:

$$R = [\ln(a/b)]/120 \quad (2)$$

where, a = absorbance at 470 nm at $t = 0$, and b = absorbance at 470 nm at $t = 120$ min. The antioxidant activity of BHT and the oils was calculated according to the following formula equation 3:

$$AA\% = [(R_{\text{control}} - R_{\text{sample/BHT}}) / R_{\text{control}}] \times 100 \quad (3)$$

where, R_{sample} and R_{control} represent the bleaching rates of β -carotene with or without addition of samples, respectively.

The oxidation rate ratio (ORR) is based on the following equation 4:

$$ORR = \frac{R_{sample}}{R_{control}} \quad (4)$$

Results and Discussion

Chemical Composition

Hydrodistillation of the *Hedychium malayanum* leaves and stems gave pale yellowish viscous oils in respective 0.038 and 0.009% yields (w/w). The leaves comprised 4.2 times more oil than the stems. GC-GC/MS analysis of the *Hedychium malayanum* leaf and stem oils has succeeded in identifying 16 and 7 compounds which comprised 98.4 and 100% of the total constituents of the respective oils. The leaf and stem oils of *H. malayanum* contained a total of 17 different chemical components (Table 1) which included 12 monoterpenes and five sesquiterpenes. The monoterpenes comprised of six hydrocarbons of α -pinene, sabinene, β -pinene, *o*-cymene, limonene and β -trans-ocimene, and other six oxygenated compounds of 1,8-cineole, borneol, 4-terpineol, α -terpineol, myrtenal and myrtenol. The five sesquiterpenes consisted of α -copaene, β -caryophyllene and β -farnesene as sesquiterpene hydrocarbons whereas longipinocarvone and caryophyllene oxide as oxygenated sesquiterpenes. It should be noted that all the components identified from the stem oil were also found in the leaf oil, except of α -terpineol which can only be found in the stem oil.

β -Pinene (39.1%), α -pinene (22.3%) and β -caryophyllene (13.7%) were the major constituents in the leaf oil while in the stem oil were β -pinene (46.7%), 1,8-cineole (17.7%) and α -pinene (16.9%). These compounds contributed 75.1 and 81.3% to the total constituent percentages of the respective leaf and stem oils. Looking at the major components in our plant oils with those from other *Hedychium* species, β -pinene (42.9%: average value of the two leaf and stem oils; also applied afterward for all the species) was also found as one of the major compounds in the oils of Malagasy *H. coronarium* (37.0%), *H. spicatum* (40.9%), Brazilian *H. coronarium* (14.2%), *H. gardinarium* (43.6%), *H. thyriforme* (23.9%), *H. eletum* (25.4%), *H. bousigoniamum* (31.3%), *H. forrestii* (14.0%), *H. flavescens* (26.7%), *H. stenopetalum* (31.8%), Vietnamese *H. coronarium* (21.8%), *H. flavum* (18.5%), *H. ellipticum* (11.4%) and French Polynesia *H. coronarium* (24.8%). It should be noted here that the *H. malayanum* oils contained higher amount of β -pinene than the other *Hedychium* species excluding *H. gardinarium*. *Hedychium stenopetalum* was rich in α -pinene (28.8%) as compared to *H. malayanum* (19.6%) used in this study. Malagasy *H. coronarium*, *H. thyriforme*, *H. eletum*, *H. bousigoniamum*, *H. coccineum*, *H. flavescens*, Vietnamese *H. coronarium*, *H. ellipticum* and French Polynesia *H. coronarium* oils also contained α -pinene as a major component but with low percentages (respective 15.6, 11.0, 16.4, 9.9, 13.5, 9.8, 10.1, 18.3 and 7.8%) than the aforementioned two oils. Another main component, 1,8-cineole, identified from *H. malayanum* was found in low concentration (11.1%) when compared with previous studies of *H. spicatum* (44.3%), Brazilian *H. coronarium* (34.8%), *H. gardinarium* (48.7%), *H. acuminatum* (76.0%), *H. larsenii* (14.4%), *H. thyriforme* (27.4%), *H. bousigoniamum* (39.3%), *H. flavescens* (12.9%), *H. flavum* (13.5%), *H. ellipticum* (40.8%) and French Polynesia *H. coronarium* (40.8%); and in slight high concentration comparing to *H. forrestii* (10.1%) and Vietnamese *H. coronarium* (10.7%). β -Caryophyllene (43.0, 13.0 and 11.1%) afforded by respective oils of Brazilian *H. coronarium*, Vietnamese *H. coronarium* and *H. flavum* was found to be more in availability than that from *H. malayanum* (9.2%). Linalool as major compound in many oils of different *Hedychium* species was not detected in the present oils.

Antioxidant Activity

DPPH free radical scavenging

In the DPPH free radical scavenging, antioxidants in the oils react with stable free radicals such as 1,1-diphenyl-2-picrylhydrazyl (deep violet color) and convert it to 1,1-diphenyl-2-picrylhydrazine with discoloration. The degree of discoloration indicates the hydrogen-donating ability of antioxidants to DPPH. Table 2 shows the mean of absorbance values and the inhibition levels of DPPH in radical scavenging test. The strength of DPPH radical scavenging activity is measured as a percentage of DPPH inhibition by antioxidants. Sacchetti et al. [4] reported that the DPPH inhibition by Trolox is between 20-30%. However, the inhibition of DPPH by Trolox in this study was

28.4%. The leaf oil of *Hedychium malayanum* showed a higher DPPH inhibition (31.1%; by 2.7%) than Trolox whereas the stem oil gave a lower one (23.9%; by 4.5%).

Table 1. Percentages of the chemical constituents of the leaf and stem oils obtained from *Hedychium malayanum*

Compound	KI	Leaf	Stem	Identification methods
Monoterpene hydrocarbons				
α -pinene	934 ^a	22.3	16.9	K, MS
Sabinene	973 ^a	2.5	-	K, MS
β -pinene	979 ^a	39.1	46.7	K, MS
<i>o</i> -cymene	1023 ^a	0.5	-	K, MS
Limonene	1029 ^a	2.3	4.1	K, MS
β - <i>trans</i> -ocimene	1058 ^a	0.9	-	K, MS
sub-total (%)		67.6	67.7	
Oxygenated monoterpenes				
1,8-cineole	1031 ^a	4.5	17.7	K, MS
Borneol	1163 ^a	0.9	-	K, MS
4-terpineol	1179 ^a	1.8	-	K, MS
α -terpineol	1191 ^a	-	4.6	K, MS
Myrtenal	1191 ^a	1.2	-	K, MS
Myrtenol	1197 ^a	1.1	-	K, MS
sub-total (%)		9.5	22.3	
Sesquiterpene hydrocarbons				
α -copaene	1386 ^a	0.7	-	K, MS
β -caryophyllene	1433 ^b	13.7	4.9	K, MS
β -farnesene	1456 ^a	0.6	-	K, MS
sub-total (%)		15.0	4.9	
Oxygenated sesquiterpenes				
Longipinocarvone	1589	0.8	-	MS
caryophyllene oxide	1600 ^c	5.5	5.1	K, MS
sub-total (%)		6.3	5.1	
Total (%)		98.4	100	

KI = Kovats index on DB-5 capillary column, K = Comparison of retention data with published data, MS = Identification based on mass spectral data, a = [17], b = [18] and c = [19].

Table 2. Absorbance values and inhibition levels of DPPH radical scavenging test

Sample	Absorbance at 517 nm	DPPH inhibition (%)
Control	1.573 ± 0.01	
Trolox	1.126 ± 0.01	28.4 ± 0.55
Leaf	1.084 ± 0.01	31.1 ± 0.46
Stem	1.197 ± 0.01	23.9 ± 0.75

Value of each data was mean ± SD (n = 3).

The high antioxidant activity of *Hedychium malayanum* leaf oil might be due to the presence of 4-terpineol (1.8%) in this oil [21]. β -Pinene, α -pinene, and β -caryophyllene were identified as the major compounds in the *Hedychium malayanum* leaf oil, but it seems that they did not contribute to the DPPH radical scavenging activity [22]. The stem oil showed weak DPPH inhibition because it possessed high content of monoterpene hydrocarbons (67.7%) [4]. Leaf oil had almost the same percentage of monoterpene hydrocarbons (67.6%), but it also contained an effective antioxidant of 4-terpineol.

β -Carotene Bleaching (BCB)

Bleaching of β -carotene in linoleic acid can be used as a measure to evaluate antioxidant activity of oils. The mechanism of β -carotene bleaching is a free radical mediated phenomenon resulting from the abstraction of a hydrogen atom from a diallylic group of linoleic acid by oxygen to form a linoleic acid free radical and a hydroperoxide. The obtained linoleic acid free radicals will then react with another oxygen to produce linoleic acid peroxides which attack the highly unsaturated β -carotene molecules. As β -carotene molecules lose their double bonds by oxidation, they lose their chromophores and characteristic orange color which can be monitored spectrophotometrically [23]. The greater the efficiency of an antioxidant, the slower will be the discoloration of β -carotene. A smaller decrease in the β -carotene absorbance, as a result of lower rate of oxidation of linoleic acid, indicated better antioxidant activity of the oils. The averages of absorbance values of the *Hedychium malayanum* oils and the synthetic antioxidant BHT against the time intervals (0 -120 min) are shown in Figure 1.

It can be observed that the smallest decrease in the β -carotene absorbance with BHT and each of the oils came between the time intervals of 90 and 120 min. The antioxidant activity percentages (AAs) and oxidation rate ratios (ORRs) were calculated at t = 120. Generally, the graph also showed that the BHT had possessed the smallest decrease in the absorbance at the time (0 – 120 min) followed by the stem oil and the leaf oil.

Variable AAs (%) and ORRs, given in Table 3, were observed for BHT and the tested oils with BHT showed the slowest rate of discoloration and thus the highest antioxidant activity (96.8%) and nonexistent oxidation rate ratio (0.00). Among the two *Hedychium malayanum* oils, the more rapid color depletion of β -carotene occurred in the presence of leaf oil with antioxidant activity of 54.7% and oxidation rate ratio of 0.38, while less decrease in absorbance of β -carotene was observed for the stem oil with good antioxidant efficacy (79.8%) and small ORR (0.20). The higher terpenic percentage in the stem oil (100%) compared to the leaf oil (98.4%) is probably the reason for its higher activity [4].

Table 3. Antioxidant activity as determined by β -carotene-linoleic acid system of BHT and *Hedychium malayanum* oils

Sample	Antioxidant activity	
	AA (%)	ORR
BHT	96.8 ± 0.21	0.00 ± 0.00
Stem	79.8 ± 1.54	0.20 ± 0.02
Leaf	54.7 ± 0.85	0.38 ± 0.01

Value of each data was mean ± SD (n = 3)

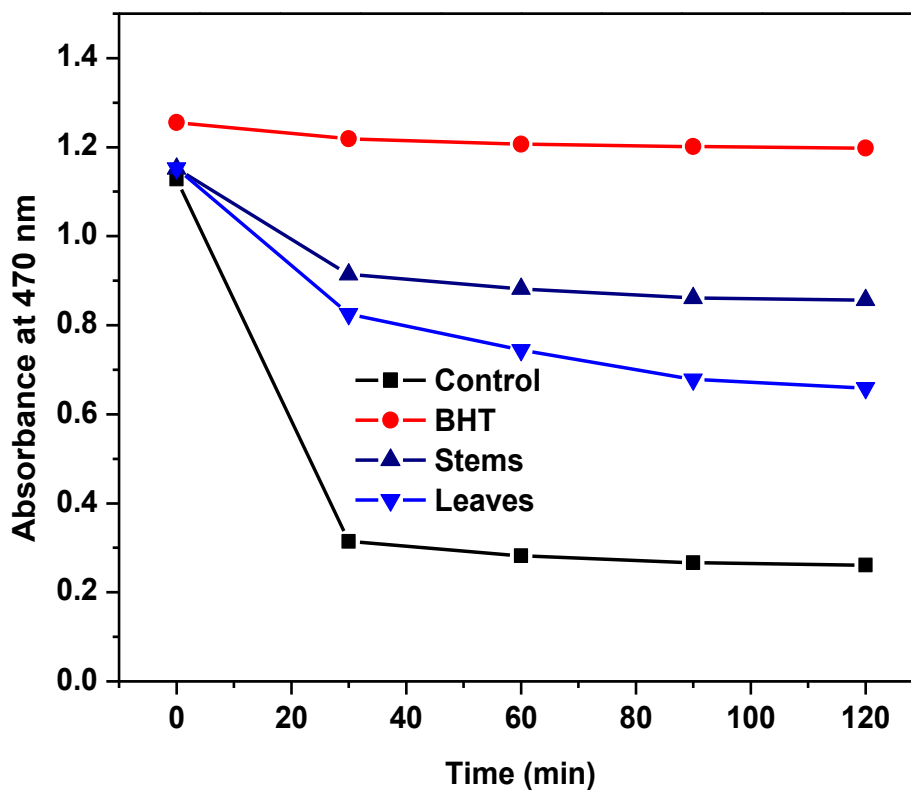


Figure 1. The averages of absorbance values of the *Hedychium malayanum* oils and the synthetic antioxidant BHT against the time intervals (0 - 120 min).

Conclusion

The identification of chemical components and evaluation of antioxidant activity of *Hedychium malayanum* essential oils were the first ever to be reported from this plant. The leaf and stem oils of *H. malayanum* possessed high DPPH inhibition and good BCB activities. Hence *H. malayanum* has the potential to be a good source for natural antioxidants which can be further utilized in cosmetic and pharmaceutical industries, and as well as natural food preservatives.

Acknowledgement

We wish to extend our gratitude to the School of Chemical Sciences and Food Technology, Universiti Kebangsaan Malaysia for the provision of laboratory facilities and technical assistance. We also wish to thank the Ministry of Higher Education Malaysia and the Universiti Kebangsaan Malaysia for financial support through the Fundamental Research Grant Scheme of UKM-ST-06-FRGS0110-2009 and Publication Intensification Fund of DLP-2013-018.

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