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Research Article

The microscopical characteristics of *Rhopalocnemis phalloides* Jungh. growing in Vietnam, as well as the phytochemical analysis and evaluation of some biological activity

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Abstract

The powder characteristics of *Rhopalocnemis phalloides* Jungh. (family Balanophoraceae) were investigated and illustrated with pictures. The phytochemical screening of *R. phalloides* showed the presence of flavonoids, tannins, organic acids. The Thin layer chromatographic method showed the presence of luteolin as the biomarker compound of *R. phalloides*. Besides, some *in vitro* biological activities of methanol extract of *R. phalloides* were evaluated. The extract of RP showed DPPH radical scavenging activity effect at concentration 100 µg/ml (SC = 63,72 ± 0,98 %) and anti-inflammatory activity at concentration 100 g/mL (I% = 67.52 ± 3.09%).

INTRODUCTION

Rhopalocnemis phalloides Jungh. (family Balanophoraceae Rich.) is a parasitic plant which distributed in the tropic and subtropic areas of southeast Asia (*Huang SM et al., 2003*). The whole plant of *Rhopalocnemis phalloides* Jungh. had been used for the treatment of the common cold and injuries from falls, and as a tonic remedy in the folk medicine of Yunnan Province, China (*Wu ZY, 2003*). This study aimed to provide the database to help identify the pharmacognosy of *Rhopalocnemis phalloides* using microscopical method and phytochemical analysis, as well as to evaluate some biological effects of this species.

MATERIALS AND METHODS

Rhopalocnemis phalloides (RP) sample was collected in April 2018, in Lamdong Province, Vietnam (Figure 1). The scientific name of *Rhopalocnemis phalloides* was assessed and stored specimens at Botanical Museum, Department of Biology, University of Natural Sciences, Hanoi National University, Hanoi, Vietnam with the voucher specimens number HNU 024070. The whole plant of *R. phalloides* were sliced and dried in an oven.

Microscopical study of material

All microscopical investigations were done using microscope Leica. The powder characteristics were investigated, described and illustrated with pictures.

Phytochemical analysis

The preliminary phytochemical sreening of *R. phalloides* were done accordingly to the procedures described in literatures (*Ngo VT et al., 2011*; *Pham TK et al., 2015*).

Extraction

The dried and powdered material of *R. phalloides* (3.7 kg) were extract with methanol (10 Litres x 3 times). The combined extracts were concentrated *in vacuo* to obtain a crude methanol residue (205 g).

Thin layer chromatography (TLC)

The 1 gram of methanol residue of *R. phalloides* was dissolved in 10 ml of methanol and filtered through a PTFE filter. Luteolin (1 g/ml) was used as a reference substance. 10 µl of each sample was applicated on TLC silica gel plate using Linomat 5 (CAMAG, Swiztland).

Plate was developed in solvent system chloroform – ethyl acetate – formic acid (5:5:1, v/v/v). The plate after development was observed and taken images in ultraviolet light at 254nm and 366nm. After that, the plate was derivatized with NP/PEG reagent and examined in ultraviolet light at 365 nm. The chromatogram was analysed to calculate the number and R_f values of tracks thanks to visionCATs software.

Biological activity

The methanol residue of *R. phalloides* was dissolved in DMSO to concentrations of 30 and 100 µg/mL.

Anti-inflammation activity

The anti-inflammatory ability was evaluated by determining the inhibitory activity of nitric oxide (NO) production on RAW 264.7 cells (*Dirsch VM, 1998*). Cardamonin was used as a positive control.

Anti-oxidant activity

The antioxidant activity was evaluated using DPPH free radical scavenging assay accordingly to the method of Shimada et al. (*Shimada K, 1992*). The percentage of scavenging effect (%) was calculated. Ascorbic acid was used as a positive control.

RESULTS

Powder characteristics of *Rhopalocnemis phalloides*

A very dark reddish-brown powder with no odour and slightly astringent taste. The diagnostic characters are: The fragments of epidermis consists of polygonal cells (1)(2). Scattered reddish-brown pigment layer (3). The fragment of supporting hair (4). The sclereids occur singly or in small group (5). The fragment of spiral vessels (6). The very occasional starch granules (7). The fragment of parenchyma composed of thin wall cells (8). (Figure 1)

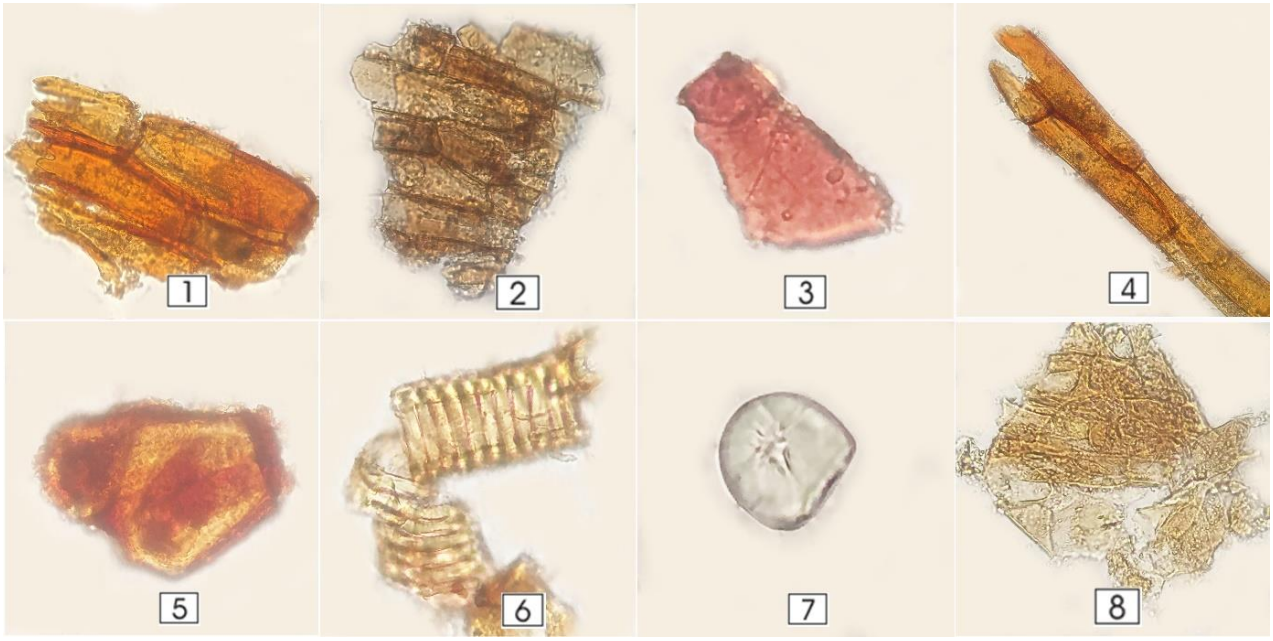

Figure 1. Powder characterristics of *Rhopalocnemis phalloides*

1,2. The fragment of epidermis; 3.Pigment layer; 4. The fragment of supporting hair; 5. The sclereids; 6. The fragment of spiral vessels; 7. Starch granules; 8. The fragment of parenchyma.

Phytochemical analysis

The preliminary phytochemical screening of *R. phalloides* showed the presence of flavonoids, tannins, organic acids and reducing sugar.

Thin layer chromatography

After derivatization, the chromatograms of *R. phalloides* possessed five zones in UV at 366 nm with Rf values were 0.06, 0.16, 0.31, 0.57, and 0.83, respectively. Among them, the yellow zone with Rf value 0.57 could be assigned as Luteolin at 366 nm. (Figure 2).

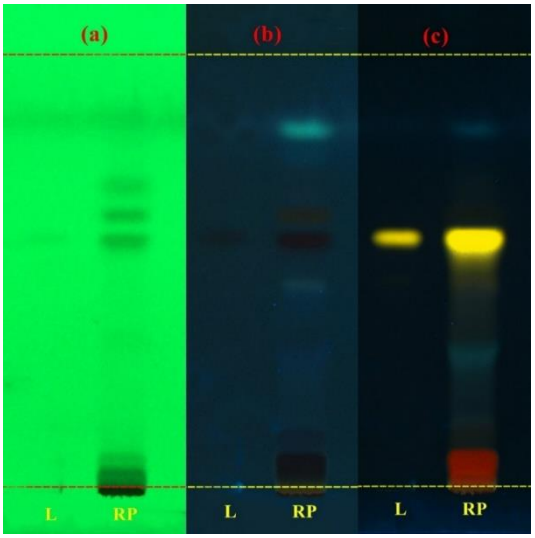

Figure 2. TLC finger printing of methanol extract of *Rhopalocnemis phalloides*
(a. observation at 254 nm, b. observation at 366 nm, c. observation at 366 nm after spraying NP/PEG reagent) (L: luteolin, RP: Rhopalocnemis phalloides)

Biological activity

The results showed that the methanol extract of *R. phalloides* possesed significant inhibitory activity on NO production in RAW264.7 cells at concentration of 100 µg/mL (Inhibition = 67.52 %). At two concentration (30 µg/mL and 100 µg/mL), the extract did not affect cell viability.

The anti-oxidant of methanol extract was evaluated through radical scavenging activity (SC %) with the DPPH assay and ascorbic acid was used as positive control. This extract had significant radical scavenging activity at concentration (100 *µ*g/ml) (SC% = 63.72 %).

Table 1. Biological activity of *R. phalloides* methanol residue

Samples	Concentration	Inhibitory activity against NO production		DPPH scavenging effect
		Inhibition ± SD (%)	Cell viability ± SD (%)	SC ± SD (%)
RP	30µg/mL	42.35 ± 1.86	95,38 ± 2.82	41.37 ± 1,32
	100µg/mL	**67.52 ± 3.09**	**84.17 ± 1.77**	**63.72 ± 0,98**
Cardamonin	0.3µM	30.25 ± 0.38	91,45 ± 0.33	
	3µM	**79.83 ± 1.01**	82,96 ± 1.37	
Ascorbic acid	10 µM			34.6
	30 µM			**72.3**

DISCUSSION

TLC analysis of *Rhopalocnemis phalloides* showed that luteolin could be detected in chromatogram. Luteolin was isolated and identified from *R. phalloides* previously (*She GM, 2010*) and this compound possessed remarkable free radical scavenging activity (SC_{50} = 16.0 ± 0.1 µM). Luteolin also exhibited some significant biological effects such as anti-inflammation, anti-allergy and anticancer (*Brown JE, 1998*; *Lien*

EJ, 1999; *Lin Y, 2008*; *Okawa, 2001*). This flavonoid could contributed to the anti-inflammatory effect and anti-oxidant effect of *Rhopalocnemis phalloides*.

CONCLUSION

The powder characteristics of *R. phalloides* were investigated and illustrated with pictures. The phytochemical analysis showed the presence of flavonoids, tannins, organic acids and reducing sugar. The TLC profiling of *R. phalloides* showed the presence of luteolin in chromatogram. These database could be useful in identification and quality control of *R. phalloides*. The methanol extract of *R. phalloides* showed significant anti-inflammatory and anti-oxidant activity.

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