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Preliminary Phytochemical Evaluation and *In Vitro* Xanthine Oxidase Inhibitory Activity of *Balanophora subcupularis* P.C. Tam and *Balanophora tobiracola* Makino (Balanophoraceae)

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ABSTRACT

Balanophora subcupularis P.C. Tam and *Balanophora tobiracola* Makino are two new recently recorded species of the genus *Balanophora* J.R. Forst and G. Forst in Viet Nam. In the present work, we investigated the phytochemical component, *in vitro* xanthine oxidase (XO) inhibitory activity of methanol extracts, fractions (n-hexane and ethyl acetate) and water residue of these two species. The Thin layer chromatography (TLC) of the methanol extracts and fractions of the two species were also done. The Ethyl acetate fractions of the two species showed the best XO inhibitory activity with IC_{50} values of 48.41 ± 1.56 µg/mL and 11.87 ± 1.28 µg/mL for *B. subcupularis* and *B. tobiracola*, respectively. The results show that the ethyl acetate fractions of the two *Balanophora* species may serve as a potential source of bioactive constituent for use in the treatment of gout.

Keywords: *Balanophora subcupularis*, *Balanophora tobiracola*, xanthin oxidase, TLC.

Introduction

Gout is a very painful medical condition in which joints are red, tender, hot and swollen. This happen when the level of uric acid in the blood is too high so that crystals get deposited from the blood and stay in joints, tendons and surrounding tissues.[1] Hyperuricemia results from the overproduction or underexcretion of uric acid. Xanthine oxidase (XO) catalyses the oxidation of xanthine and hypoxanthine into uric acid. XO inhibitors which block the terminal step in uric acid biosynthesis could lower the uric acid concentration in plasma so that they are potential to be employed for the treatment of gout.[2]

In the sixteen genera of the family Balanophoraceae, chemical composition of the genus *Balanophora* J.R. Forst & G. Forst have been investigated and were found to contain mostly phenylpropanoids, lignans, flavonoids, triterpenoids, and sterols. Antioxidant, anti-inflammatory and hypouricemic effects have been reported from *Balanophora* spp.[3,4] In the genus *Balanophora* J.R. Forst & G. Forst, the species *B. laxiflora* Hemsl showed good XO inhibitory activity[4] while other species of this genus have not been studied for their XO inhibitory activity. *Balanophora subcupularis* PC. Tam and *Balanophora tobiracola* Makino are two new recorded species of the genus *Balanophora* from the Flora of Viet Nam[5,6] and there is no study on the XO inhibitory activity of these two species. This paper provided database on the phytochemical screening, Thin layer chromatography and *in vitro* XO inhibitory activity of the methanol extracts and fractions of *B. subcupularis* and *B. tobiracola* from Viet Nam.

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Materials and Methods

Chemicals

Chemical and reagents for phytochemical screening were of analytical grade. Reference compounds (gallic acid, caffeic acid, and quercetin) were purchased from Chemfaces (China). The TLC was performed on TLC plates silica gel 60 F_{254} (20 cm x 10 cm), and were purchased from Merck (Germany). Xanthine oxidase and xanthine were obtained from Sigma Aldrich Chemicals.

Plant materials

The fresh whole plants of *B. subcupularis* were collected in Lam Dong province, Viet Nam in November 2016, while the fresh whole plants of *B. tobiracola* were collected in Lang Son province, Viet Nam in January 2018. The specimens of the two species were preserved in the herbarium of the Museum of Biology, Faculty of Biology, University of Science, Vietnam National University with the voucher specimen numbers HNU 022609 and HNU 024056 for *B. subcupularis* and *B. tobiracola*, respectively.

Preparation of samples

The air-dried powdered whole plants (200 g each) of the two species were reflux extracted with methanol (0.5 L, 3 times). After filtration, the filtrates were concentrated using a rotary evaporator (Buchi Rotavapor R-200). The methanol extracts of the two species were suspended in distilled water (200 mL) and successively partitioned with n-hexane and ethyl acetate to give n-hexane fractions, ethyl acetate fractions and water residues.

Phytochemical screening

Phytochemical screening of the methanol extracts, n-hexane fractions, ethyl acetate fractions and water residues of the two *Balanophora* species were carried out. The presence of flavonoids, coumarins, tannins, anthranoid, alkaloids, and sterols were tested for according to standard procedures.[7,8]

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Figure 1: The whole plants of two new recorded species of the genus *Balanophora* J.R. Forst & G. Forst in Viet Nam.

Thin layer chromatography

The methanol extracts, n-hexane fractions, ethyl acetate fractions and water residues of the two species were dissolved in methanol to make a concentration of 25 mg/mL. After filtration, they were used for Thin layer chromatographic (TLC) analysis. Two main phenolic acids in *Balanophora* species, gallic acid and caffeic acid were used as reference compound for TLC. These compounds were dissolved in methanol to make a concentration 1 mg/mL. Separation and qualitative analysis of samples and reference compounds were performed using a CAMAG (Switzerland) HPTLC system equipped with Linomat V applicator, ADC 2 Automatic Developing Chamber, CAMAG TLC Visualizer and visionCATs software. TLC development was carried out in a CAMAG twin-trough chamber (20 cm × 10 cm) which was pre-saturated with 25-mL mobile phase Chloroform – Ethyl acetate – Formic acid (5:5:1) at room temperature (25°C ± 2°C) for 20 min. The length of the chromatogram run was 8 cm, and the TLC plates were visualized at λ = 254 nm and λ = 366 nm in CAMAG TLC Visualizer before being derivatized with Natural products-polyethylene glycol reagent (NP/PEG) and visualized at λ = 366 nm.

Assay of XO inhibitory activity

The XO inhibitory activity was assayed spectrophotometrically base on the procedures reported by Noro *et al*.[9] and Nguyen *et al*.[10] with modifications. Costar 96-well plates (Corning) and ELISA system (Biotek microplate reader and Awareness incubator shaker) were used. The assay mixture consisting of 50 µL of test solution (300 µg/mL, 100 µg/mL, 50 µg/mL, 30 µg/mL, 10 µg/mL, 3 µg/mL), 35 µL of 70 mM phosphate buffer (pH 7.5), and 30 µL of enzyme solution (0.01 units/mL in 70 mM phosphate buffer, pH 7.5) was prepared immediately before use. After preincubation at 15°C for 15 min, the reaction was initiated by the addition of 60 µL of substrate solution (150 µM xanthine in the same buffer). The assay mixture was incubated at 25°C for 30 min. The reaction was stopped by adding 25 µL of 1N HCl, and the absorbance was measured at 290 nm. A blank was prepared in the same way, but the enzyme solution was added to the assay mixture after adding 1N HCl. One unit of XO is defined as the amount of enzyme required to produce 1 µmol of uric acid/min at 25°C.

XO inhibitory activity was expressed as the percentage inhibition of XO in the above assay system, calculated as (1 – B/A) x 100, where A and B are the activities of the enzyme without and with test material, respectively. The IC_{50} values were calculated from the mean values of data from four determinations. The extracts were dissolved initially in DMSO followed by dilution with the buffer such that the final concentration of DMSO was less than 0.25%. Quercetin (30 µg/mL, 10 µg/mL, 7.5 µg/mL, 5 µg/mL, 2.5 µg/mL), a known inhibitor of XO, was used as a positive control.

Results and Discussion

Phytochemical screening

The results of phytochemical screening of methanol extracts, n-hexane fractions, ethyl acetate fractions and water residues of the two *Balanophora* species are shown in table 1.

Thin layer chromatography

Figure 2 showed the TLC chromatogram of methanol extracts, n-hexane fractions, ethyl acetate fractions and water residues of the two species and reference compounds (gallic acid and caffeic acid). The densitogram of the samples and reference compounds were evaluated with visionCATs software.

Table 1: Photochemical screening of two *Balanophora* species.

Chemical components	*B. tobiracola*				*B. subcupularis*			
	MeOH extract	n-hexane fraction	EtOAc fraction	Water residue	MeOH extract	n-hexane fraction	EtOAc fraction	Water residue
Cardiac glycosides	-	-	-	-	-	-	-	-
Flavonoids	+	-	+	+	+	-	+	+
Coumarins	+	+	+	+	+	+	+	+
Tannins	+	+	+	+	+	+	+	+
Saponin	-	-	-	-	-	-	-	-
Steroids	+	+	+	-	+	+	+	-

(+): indicate present, **(-):** indicate absent of component.

Table 2. *In vitro* xanthine oxidase inhibitory activity of *B. subcupularis* and *B. tobiracola* extracts and fractions.

Samples	(IC_{50} µg/mL)				
	Methanol extract	n-hexane fraction	Ethyl acetate fraction	Water residue	Control (Quercetin)
Balanophorasub cupularis	88.67 ± 1.22	103.10 ± 4.32	48.41 ± 1.56	93.09 ± 1.19	4.80 ± 1.07
Balanophora tobiracola	118.5 ± 1.09	87.45 ± 1.30	11.87 ± 1.28	115.50 ± 18.42	

Data are expressed as mean ± SD of four determinations.

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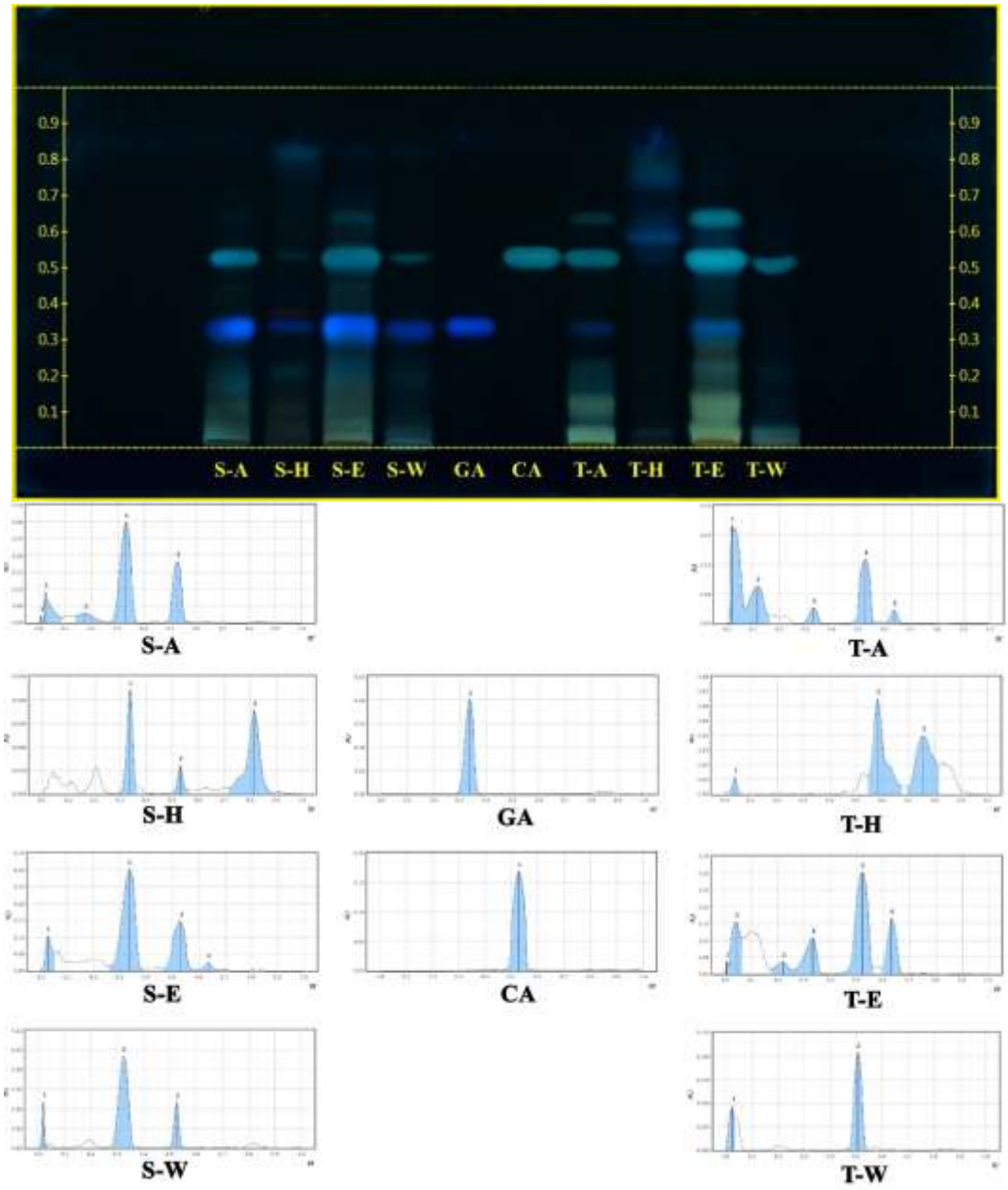

Figure 2: Thin layer chromatogram and densitogram of methanol extracts and fractions of two species developed with the solvent system Chloroform – Ethyl acetate – formic acid (5:5:1) observed at λ = 366nm, after being derivatized with NP/PEG reagent.
S-A: Methanol extract of *B. subcupularis*, **S-H:** n-hexane fraction of *B. subcupularis*, **S-E:** Ethyl acetate fraction of *B. subcupularis*, **S-W:** Water residue of *B. subcupularis*, **GA:** gallic acid, **CA:** caffeic acid, **T-A:** Methanol extract of *B. tobiracola*, **T-H:** n-hexane fraction of *B. tobiracola*, **T-E:** Ethyl acetate fraction of *B. tobiracola*., **T-W:** Water residue of *B. tobiracola*,

The TLC chromatograms of the extracts (methanol extracts, n-hexane fractions, ethyl acetate fractions and water residues) of the two *Balanophora* species have some similarities and dissimilarities when visualized at λ = 254nm, λ = 366 nm and after derivatization with NP/PEG reagent. The ethyl acetate fractions of the two species have spots with high fluorescence intensity which were equivalent to the reference compounds (gallic acid spot has R*f*-value = 0.263, caffeic acid spot has R*f*-value = 0.439) while equivalent spots in n-hexan, water residues of two species have lower fluorescence intensities.

Xanthin oxidase inhibitory activity
The results of the *in vitro* XO inhibitory activity of test samples and control is shown in table 2.
Ethyl acetate fractions of *B. subcupularis* and *B. tobiracola* showed good *in vitro* XO inhibitory activity with IC_{50} value of 48.41 ± 1.56 µg/mL and 11.87 ± 1.28 µg/mL, respectively.

The phytochemical screening showed that the two *Balanophora* species are rich in polyphenols. The TLC chromatogram of the two species showed that phenolic compounds such as gallic acid, and caffeic acid which possess good xanthine oxidase inhibitory activity[2,11] are mostly concentrated in ethyl acetate fractions. This correlates with the results of xanthine oxidase inhibitory assay in which the ethyl acetate fractions of the two *Balanophora* species possessed the highest activity.

Conclusion

The present study has shown the ethyl acetate fractions of the two *Balanophora* species to possess *in vitro* xanthine oxidase inhibitory activity with IC_{50} values of 48.41 ± 1.56 µg/mL and 11.87 ± 1.28 µg/mL for *B. subcupularis* PC. Tam and *Balanophora tobiracola* Makino, respectively. Further research work should be focused on the *in vivo* xanthine oxidase inhibitory activity as well as isolation of compounds

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from the two species, especially xanthine oxidase inhibitors from the ethyl acetate fractions.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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References

1. Eggebeen AT. Gout: an update. Am Fam Physician 2007; 76 (6):801–808.
2. Lespade L and Bercion S. Theoretical Study of the Mechanism of Inhibition of Xanthine Oxydase by Flavonoids and Gallic Acid Derivatives. J Phys Chem B. 2010; 114:921–928.
3. Wang X, Liu Z, Qiao W, Cheng R, Liu B, She G. Phytochemicals and biological studies of plants from the genus Balanophora. Chem Cent J. 2012; 6:79.
4. Ho S-T. Tung Y-T, Huang C-C, Kuo C-L, Lin C-C, Yang S-C, Wu J-H. "The Hypouricemic Effect of *Balanophora laxiflora* Extracts and Derived Phytochemicals in Hyperuricemic Mice". Evid Based Compl Altern Med. 2012; 2012:1–7.
5. Nguyen T-T, Nguyen V-T, Nguyen Q-H. First record of *Balanophora tobiracola* Makino (Balanophoraceae) from Viet Nam. Biosci Discov. 2018; 9(2):297-301.
6. Tung NT, Than NV, Hung NQ. *Balanophora subcupularis* P. C. Tam (Balanophoraceae): New Record Species for Flora of Vietnam. J Pharmacogn Nat Prod. 2017; 3:142.
7. Thu NV and Hung T. "Dược liệu học", Vol. 1, Medical Publishing House, 2011.
8. Pham Thanh Ky et al. "Dược liệu học", Vol. 2, Medical Publishing House. 2015.
9. Noro T, Oda Y, Miyase T, Ueno A, Fukushima S. Inhibitor of xanthin oxidase from flowers and buds of Daphne genkwa, Chem Pharm Bull. 1983; 31(11):3984-3987.
10. Nguyen MT, Awale S, Tezuka Y, Tran QL, Watanabe H, Kadota S. Xanthin oxidase inhibitory activity of Vietnamese Medicinal plants. Biol Pharm Bull. 2004; 27(9):1414–1421.
11. Masuda T, Shingai Y, Takahashi C, Inai M, Miura Y, Honda S, Masuda A. Identification of a potent xanthine oxidase inhibitor from oxidation of caffeic acid. Free Rad Bio Med. 2014; 69:300–307.