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

Nguyen T. Tung1*, Nguyen V. Quan1, Nong P. Anh1, Nguyen V. Phuong1, Nguyen Q. Hung2

1Ha Noi University of Pharmacy, 15 Le Thanh Tong, Hoan Kiem, Ha Noi, Viet Nam.
2Institute of Ecology and Biological Resources (IEBR), Vietnam Academy of Science and Technology, Hanoi, Vietnam (VAST), 18 Hoang Quoc Viet, Cau Giay, Ha Noi, Viet Nam.

**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 IC50 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. 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.

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.1,3 In the genus *Balanophora* J.R. Forst & G. Forst, the species *B. laxiflora* Hemsl showed good XO inhibitory activity4 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 Nam5,6 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.

*Corresponding author. E-mail: thanhthuong.pharmacist@gmail.com Tel: +84975002697*

Citation: Tung NT, Quan NV, Anh NP, Phuong NV, Hung NQ. Preliminary Phytochemical Evaluation and *In Vitro* Xanthine Oxidase Inhibitory Activity of *Balanophora subcupularis* P.C. Tam and *Balanophora tobiracola* Makino (Balanophoraceae). Trop J Nat Prod Res. 2019; 3(1):6-9. doi:10.26538/tjpnpr/v3i1.2

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

**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 F254 (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

---

*Corresponding author. E-mail: thanhthuong.pharmacist@gmail.com Tel: +84975002697*
**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.

**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.

<table>
<thead>
<tr>
<th>Chemical components</th>
<th><em>B. tobiracola</em></th>
<th><em>B. subcupularis</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MeOH fraction</td>
<td>EtOAc fraction</td>
</tr>
<tr>
<td>Cardiac glycosides</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Coumarins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Saponin</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

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

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

<table>
<thead>
<tr>
<th>Samples</th>
<th>(IC₅₀ µg/mL)</th>
<th>MeOH extract</th>
<th>n-hexane fraction</th>
<th>Ethyl acetate fraction</th>
<th>Water residue</th>
<th>Control (Quercetin)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Balanophora subcupularis</em></td>
<td>88.67 ± 1.22</td>
<td>103.10 ± 4.32</td>
<td>48.41 ± 1.56</td>
<td>93.09 ± 1.19</td>
<td></td>
<td>4.80 ± 1.07</td>
</tr>
<tr>
<td><em>Balanophora tobiracola</em></td>
<td>118.5 ± 1.09</td>
<td>87.45 ± 1.30</td>
<td>11.87 ± 1.28</td>
<td>115.50 ± 18.42</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SD of four determinations.
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.


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 Rf-value = 0.263, caffeic acid spot has R-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 IC50 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 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 IC50 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.
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.

Acknowledgements
The authors wish to appreciate the financial support from Hanoi University of Pharmacy. They also express their sincere thanks to VAST for their help in collecting Balanophora materials through the Project VAST.CTG.04/16-17.

References