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# Antimalarial alkaloids from a Bhutanese traditional medicinal plant Corydalis dubia

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## Abstract

Ethnopharmacological relevance: Corydalis dubia is used in Bhutanese traditional medicine as a febrifuge and for treating infections in the blood, liver and bile which correlate to the signs and symptoms of malarial and microbial infections.

Aim of the study: To validate the ethnopharmacological uses of the plant and to discover potential new therapeutic drug leads. Materials and methods C. dubia was collected from Bhutan and the alkaloids were obtained using acid–base fractionation and separation by repeated column and preparative plate chromatography. The alkaloids were identified from analysis of their physiochemical and spectroscopic data and were tested for antiplasmodial, antimicrobial and cytotoxicity activities.

Results: A systematic extraction and isolation protocol yielded one new natural product, dubiamine, and seven known isoquinoline alkaloids, scoulerine, cheilanthifoline, protopine, capnoidine, bicuculline, corydecumbine and hydrastine. Among the four alkaloids tested, scoulerine showed the best antiplasmodial activity with IC50 values of 5.4 μM and 3.1 μM against the antifolate sensitive and the multidrug resistant P. falciparum strains: TM4/8.2 and K1CB1, respectively. None of the alkaloids tested showed significant antimicrobial or cytotoxicity activities.

Conclusions: The antiplasmodial test results, of the isolated alkaloid components, are commensurated with the ethnopharmacological uses of this plant.



## Keywords

alkaloids, bhutanese, traditional, antimalarial, medicinal, dubia, plant, corydalis, CMMB


## Disciplines

Life Sciences | Physical Sciences and Mathematics | Social and Behavioral Sciences


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**Antimalarial alkaloids from a Bhutanese traditional medicinal plant *Corydalis dubia***

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ABSTRACT

*Ethnopharmacological relevance: Corydalis dubia* is used in Bhutanese traditional medicine as a febrifuge and for treating infections in the blood, liver and bile which correlate to the signs and symptoms of malarial and microbial infections.

*Aim of the study:* To validate the ethnopharmacological uses of the plant and to discover potential new therapeutic drug leads.

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alkaloids were identified from analysis of their physiochemical and spectroscopic data and were tested for antiplasmodial, antimicrobial and cytotoxicity activities.

*Results:* A systematic extraction and isolation protocol yielded one new natural product, dubiamine, and seven known isoquinoline alkaloids, scoulerine, cheilanthifoline, protopine, capnoidine, bicuculline, corydecumbine and hydrastine. Among the four alkaloids tested, scoulerine showed the best antiplasmodial activity with $IC_{50}$ values of 5.4 μM and 3.1 μM against the antifolate sensitive and the multidrug resistant *P. falciparum* strains: TM4/8.2 and K1CB1, respectively. None of the alkaloids tested showed significant antimicrobial or cytotoxicity activities.

*Conclusions:* The antiplasmodial test results, of the isolated alkaloid components, are commensurated with the ethnopharmacological uses of this plant and generated a new antiplasmodial drug lead.

*Keywords*

Bhutanese traditional medicine; medicinal plant, *Corydalis dubia*; antimalarial; scoulerine

## 1. Introduction

*Corydalis dubia* Prain (Fumariaceae), which is locally known as *Re-skon*, is an annual yellow-flowering glabrous herb of lower stature arising from tuberous rootstock. This plant is endemic to Bhutan, Sikkim (India) and Chumbi Valley of Tibet (Grierson and Long, 1984). In Bhutan, it occurs in the wild in localized parts of Lingzhi and Bumthang region at an altitude range of 4294-5039 meters above sea level (Wangchuk et al., 2008; Anonymous, 2008). It grows sparsely with recorded density of 0.6 to 3.6 plants/$m^2$ (Anonymous, 2008). Therapeutic uses of this plant were reported for detoxifying impure blood, treating fever arising from the infections of liver, heart, lung, pancreas and kidney and alleviating neuralgia and complicated disorders that have resulted from a combination of defective air, bile and phlegm (Wangchuk et al., 2011). These are symptoms which correlate to both malarial and microbial infections. A preliminary phytochemical screening demonstrated the presence of alkaloids

and that the crude MeOH and $CHCl_3$ alkaloid extracts showed significant antiplasmodial activity against chloroquine and antifolate resistant *Plasmodium falciparum* strains: TM4/8.2 and K1CB1 (Wangchuk et al., 2011). This prompted us to investigate the alkaloid constituents of *C. dubia* with a view to generate potential new therapeutic agents and at the same time validate the use of this plant in Bhutanese traditional medicine (BTM) for the treatment of malarial or microbial infections.

A systematic extraction and isolation protocol yielded one new natural product which we named as dubiamine (**1**) drawn from the species name of the plant. Seven known isoquinoline alkaloids were also isolated which were identified as scoulerine (**2**), cheilanthifoline (**3**), protopine (**4**), capnoidine (**5**), bicuculline (**6**), corydecumbine (**7**) and hydrastine (**8**). Compounds **1**, **2**, **5** and **6** were investigated for their antiplasmodial, antimicrobial and cytotoxicity activities. Compounds **2** showed significant antiplasmodial activity which is reported here for the first time. The antiplasmodial activities of compounds **3** and **4** were reported earlier by us and are included in Table 1.

## 2. Materials and methods

### *2.1. Plant material*

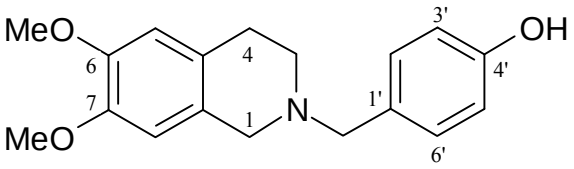
Wild *C. dubia* (whole part) was collected from Thruenchela (Altitude: 4651 m; Latitude: 27°56' 00.1"; Longitude: 89° 26' 11.6"; global positioning system (GPS) point number: 167; Site number: P167; Slope: 30°; Aspect: North-West), under the Lingzhi region in Bhutan in August 2009. The herbarium voucher specimen with accession number 78 was authenticated by Samten, a pharmacognosist at the Pharmaceutical and Research Unit (PRU) and was deposited at the herbarium unit of PRU, Thimphu, Bhutan.

### *2.2. Extraction, isolation and identification of alkaloids*

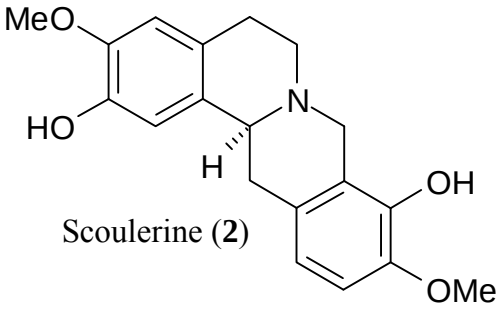
The extraction and separation of the alkaloids from the crude methanol extract (65.12 g obtained from 2 kg dry plant material) was undertaken via an acid/base extraction procedure using the analytical chemicals and tools as reported in Wangchuk et al. (2012). This protocol furnished the $CH_2Cl_2$ extract (17.87 g) which contained a mixture of acidic and neutral compounds and the $CHCl_3$ extract (1.13 g) which contained the basic alkaloids. Focusing on the alkaloids, the $CHCl_3$ extract (1.2 g) was chromatographed on a silica gel (120 g, 200-300 mesh) column, eluting with a gradient solvent system of MeOH-$CHCl_3$ (total combined volume of 200 mL in a v/v ratio of 0:100, 0.5:99.5, 1.5:98.5, 3:97, 4:96, 5:95, 7:93, 10:90, 15:85, 20:80, 30:70, 40:60) to obtain fractions F1-F11. Fraction F4, upon crystallization from $CHCl_3$/MeOH (1:1), yielded compound **5** (80 mg). Its mother liquor was purified by column chromatography eluting with a gradient solvent system of MeOH-$CHCl_3$ to obtain five sub-fractions F4.1-F4.5. The separation of these sub-fractions F4.2, F4.3, F4.4 and F4.5 using preparative TLC plates (mobile phase; $CHCl_3$/ethyl acetate/$NH_4OH$) (55:45:4 drops) yielded compounds **3** (15 mg), **6** (18 mg), **7** (5 mg)**,** and **8** (0.7 mg). Fractions 5 and 6 were combined (F56) and then separated by the column chromatography with a gradient solvent system of ethyl acetate-$CHCl_3$ (50 mL, v/v ratio of 0:100, 20:80, 40:60, 50:50, 60:40, 80:20, 100:0) to obtain sub-fractions F56.1-F56.6. Further separation of sub-fraction F56.2 on preparative TLC plates (mobile phase; $CHCl_3$/ethyl acetate (55:45)) gave compound **2** (9 mg). Crystallization of the sub-fraction F56.3 from $CHCl_3$/MeOH (1:1) furnished compound **1** (7 mg). Fraction F7 was crystallized from the ethyl acetate/hexane (1:1) to give compound **4** (160 mg), the major alkaloid of the plant.

Compound **1** was identified as a new natural product which we named as dubiamine after the name of the plant species. It was obtained as colorless needle-like crystals with mp 192.8-194.6 °C; ESI-MS (*m/z*) 300 [M + $H^+$]; EI-MS (*m/z*): 299 [$M^{+\bullet}$], 298, 192, 176, 164, 148 (100%), 134, 121, 107; and HR-ESI-MS (*m/z*) 300.1597 [M + H]$^+$ (300.1600 calc. for $C_{18}H_{22}NO_3$). $^1$H-NMR (500 MHz, $CDCl_3$): δ 2.73 (2H, *t*, *J* = *5.8* Hz, H-3), 2.81 (2H, *t*, *J* = *5.8* Hz, H-4), 3.53 (2H, *s*, H-1), 3.60 (2H, *s*,

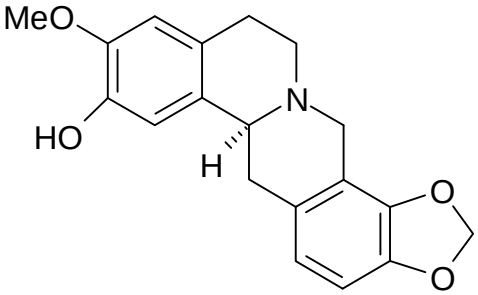
H-2), 3.80 (3H, *s*, OMe on C-7), 3.83 (3H, *s*, OMe on C-6), 6.48 (1H, *s*, H-8), 6.58 (1H, *s*, H-5), 6.75 (2H, *d*, *J* = 8.0 Hz, H-3' and H-5'), 7.22 (2H, *d*, *J* = 7.5 Hz, H-2' and H-6'). $^{13}$C-NMR (125 MHz, $CDCl_3$): δ 28.7 (C-4), 50.7 (C-3), 55.6 (C-1), 56.0 (OMe), 56.1 (OMe), 62.2 (C-2), 109.7 (C-8), 111.6 (C-5), 115.3 (C-3' and C-5'), 126.3 (C-8a), 126.7 (C-4a), 130.1 (C-1'), 130.7 (C-2' and C-6'), 147.6 (C-6 and C-7).



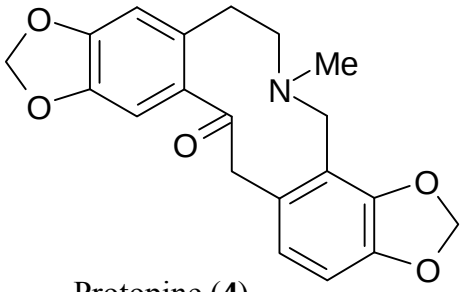

Dubiamine (**1**)



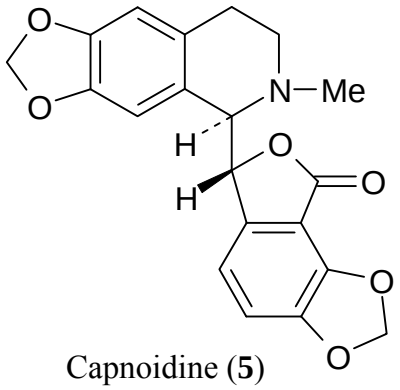

Scoulerine (**2**)



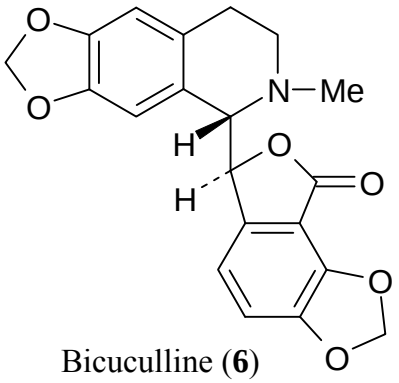

Cheilanthifoline (**3**)



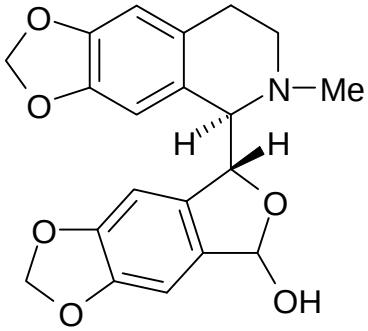

Protopine (**4**)



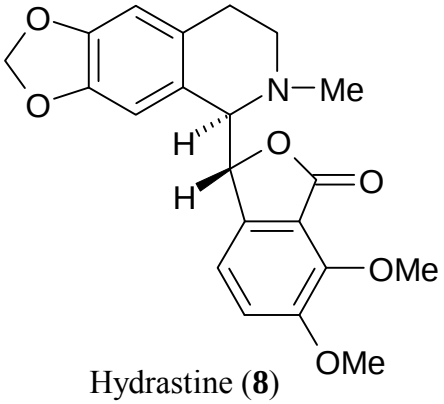

Capnoidine (**5**)




Bicuculline (**6**)




Corydecumbine (**7**)




Hydrastine (**8**)

**Fig. 1.** Alkaloids isolated from *Corydalis dubia*.

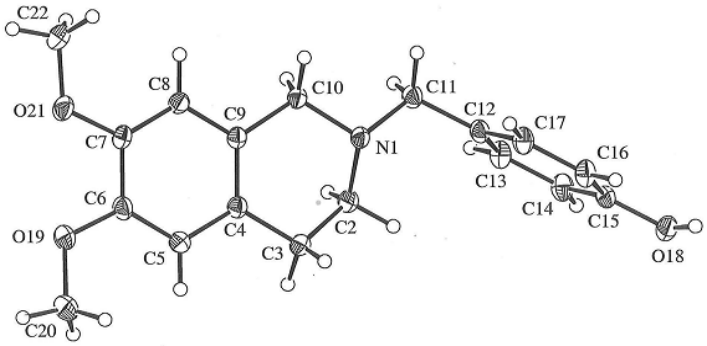



**Fig. 2.** Single crystal structure of dubiamine (**1**) with CCDC 872799.

The structure of **1** (Fig. 1) was confirmed from the analysis of its single crystal X-ray crystallographic data (Fig. 2): ($C_{18}H_{21}NO_3$), $M_r$ 299.37. Monoclinic, $P2_1/n$, a = 6.4140 (1) Å, b = 13.0324 (3) Å, c = 19.2120 (4) Å, β = 98.8791 (11)°, V = 1586.68 (6) Å$^3$, Z = 4, F (000) = 640, $D_x$ = 1.253 Mg m$^{-3}$, Mo*K*α radiation, λ = 0.71073 Å, cell parameters from 26826 reflections, θ = 26-27.5°, μ = 0.09 mm$^{-1}$, specimen = 0.42 × 0.35 × 0.22 mm (colorless block). 46844 reflections were measured to $2\theta_{max}$ = 55° and merged to 3625 unique reflections. Final *R*(3174 reflns with $F^2 > 2\sigma(F^2)$) = 0.037, wR (all data) = 0.101, S = 0.99.

The crystal structure has been deposited at the Cambridge Crystallographic Data Centre and allocated the deposition number CCDC 872799.

While **1** has not been reported as a natural product, it has been synthesized *in vitro* from the enzymatic oxidation of *d*,*l*-*N*-norarmepavine which was assigned the IUPAC name as 4-((6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)methyl)phenol (Inubushi et al., 1969). This alkaloid was closely related to sendaverine and its derivatives (Pandey and Tiwari, 1980; Kametani and Ohkubo, 1965; Suau et al., 1994). Other known compounds **2**-**8** were identified through MS library matching techniques (NIST08s) and subsequent comparison of their melting point, optical rotation, mass spectra and NMR spectra ($^1$H and $^{13}$C) with those reported. Compound **2** was identified as scoulerine (Brochman-Hanssen and Nielson 1966; Cheng et al., 2008), compound **3** as cheilanthifoline (Okamoto

et al., 1971; Haisova and Slavik, 1973; Yeola and Mali, 1984; Rucker et al., 1994; Cheng et al., 2008), compound **4** as protopine (Abou-Donia et al., 1980; Takahashi et al., 1985; Seger et al., 2004), compound **5** as capnoidine (Blasko et al., 1982; Ribar and Meszaros, 1991; Pilar et al., 2010) whose structure was also confirmed from a X-ray crystal structure (not shown here), compound **6** as bicuculline (Edwards and Handa, 1961; Abou-Donia et al., 1980; Blasko et al., 1982; Elango, et al., 1982; Basha et al., 2002), compound **7** as corydecumbine (Basnet et al., 1993) and compound **8** as hydrastine (Blasko et al., 1982; Elango, et al., 1982).

### *2.3. Bioassay methods*

Antiplasmodial, antimicrobial and cytotoxicity bioassays were carried out using the standard protocols reported by Wangchuk et al., (2011). The test strains of *Plasmodium falciparum* used for the antiplasmodial assay were K1CB1, a multidrug resistant strain; and TM4/8.2, a wild type chloroquine and antifolate sensitive strain. Chloroquine, cycloguanil and pyrimethamine were used as reference drugs or positive controls in the antiplasmodial assays. DMSO (0.1%) and distilled water were used as controls to rule out the solvent effects on the bioassay results of the test samples. All the experiments were performed three times in duplicate (3x2).

## 3. Results and Discussions

Given the traditional use of *C. dubia* to treat fever and various disorders bearing relevance to malaria and microbial infections and the evidence that the crude alkaloid extracts exhibited significant antiplasmodial activity (Wangchuk et al., 2011), we investigated the bioactive alkaloid constituents of this plant. Out of eight alkaloids (compounds **1**-**8**, Fig. 1) isolated from this plant, we examined compounds **1**, **2**, **5** and **6** for their antiplasmodial, cytotoxicity and antimicrobial and activities (Table 1).

**Table 1**

Antiplasmodial activity ($IC_{50}$ in µg/mL) of the pure compounds **1**, **2**, **5** and **6** isolated from *C. dubia.*

| Samples | Antiplasmodial | | Cytotoxicity | |
|---|---|---|---|---|
| | TM4/8.2 | K1CB1 | Vero cells | KB cells |
| MeOH extract | 6.38 ± 0.62[a] | 9.50 ± 0.84[a] | >10 [a] | >10 [a] |
| $CHCl_3$ alkaloid extract | 2.21 ± 0.43 [a] | 2.84 ± 0.32 [a] | >10 [a] | >10 [a] |
| Dubiamine (**1**) | >20 | >20 | >20 | >20 |
| Scoulerine (**2**) | 1.78 ± 0.72 | 1.04 ± 0.08 | >20 | >20 |
| Cheilanthifoline (**3**) | 0.90 ± 0.27[b] | 1.22 ± 0.84[b] | Not tested | Not tested |
| Protopine (**4**) | 1.45 ± 0.53[c] | 1.38 ± 0.31[c] | >3.5[c] | >3.5[c] |
| Capnoidine (**5**) | >20 | >20 | >20 | >20 |
| Bicuculline (**6**) | >10 | >10 | >10 | >10 |
| Chloroquine[d] | 0.010 | 0.089 | | |
| Cycloguanil[d] | 0.009 | 0.810 | | |
| Pyrimethamine[d] | 0.020 | 7.700 | | |
| Ellipticine[e] | | | 0.093 | |
| Doxorubicin[e] | | | | 0.56 |

[a] original activity taken from Wangchuk et al. (2011).

[b] original activity taken from Wangchuk et al. (2010).

[c] original activity taken from Wangchuk et al. (2012).

[d] Reference drugs for antiplasmodial activity.

[e] Reference drugs for cytotoxicity activity.

Interestingly, compound **2** demonstrated significant *in vitro* antiplasmodial activity against the *P. falciparum* strains: TM4/8.2 (a wild type chloroquine and antifolate sensitive strain) and K1CB1 (multidrug resistant strain) with $IC_{50}$ values of 1.78 µg/mL (5.47 µM) and 1.04 µg/mL (3.1 µM), respectively. Compounds **3** and **4** were isolated from different plant species and their antiplasmodial activities were determined earlier by us (Wangchuk et al., 2010; Wangchuk et al., 2012) and are included in this study (Table 1). Compounds **2**, **3**, and **4** exhibited similar antiplasmodial activity**.** However, the activities of compound **2** were found to be almost two fold better than the $CHCl_3$ crude alkaloid extract (2.21 and 2.84 µg/mL against TM4/8.2 and K1CB1 strains, respectively); seven fold

better than the crude MeOH extract (6.38 μg/mL for TM4/8.2 and 9.50 μg/mL for K1CB1 strains); and eight fold better than the conventional antimalarial drug pyrimethamine (reference drug) against the K1CB1 strain (Table 1). Although the antiplasmodial activity of scoulerine (**2**) does not meet the criteria of the medicines for malaria venture (MMV), it represents a potential new scaffold based on which the improved antimalarial lead compounds can be drawn, particularly as it did not show mammalian cell line (KB and Vero cells) toxicity (Table 1) and has not been previously reported. Scoulerine has been reported to exhibit other activities such as: anti-topoisomerase I (Cheng et al., 2008), $GABA_A$ receptor agonist (Eisenreich et al., 2003), and antiemetic, antitussive and antibacterial activities (Taylor and Francis, 2011).

Compounds **1**, **5** and **6** did not show antiplasmodial activity even at the highest tested concentration of 10-20 μM. Due to the smaller quantities of compounds **7** and **8**, their biological activity studies were not carried out.

Since compounds **2**, **3** and **4** have shown significant antiplasmodial activities and that protopine (**4**) was the major alkaloid present, it may be deduced that these three alkaloids (alone or synergistically) may be responsible for the significant activity of the $CHCl_3$ crude alkaloid extract of this plant. The antimicrobial assay of the pure alkaloids (**1**-**6**) did not identify any compounds with significant activity against *Escherrichia coli*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *MRSA, Bacillus subtilis*, *Vibrio cholera* and *Candida albicans* with all showing MIC values greater than 250 μg/mL.

The significant antiplasmodial activities of the crude extracts and the isolated three pure compounds **2**, **3** and **4** provide support for the use of *C. dubia* in BTM for treating fever associated with malaria and other infections of the blood, liver and bile. Thus, our study verified the ethnomalarial uses of the plant.

## 4. Conclusions and future directions

In summary, our study found the following: a) a new natural product (**1**) was isolated which we named as dubiamine and its structure was confirmed from its single crystal X-ray structure b) compound **2** showed significant *in vitro* antiplasmodial activity without mammalian cell toxicity and was identified as a potential antimalarial hit scaffold c) the *in vitro* antiplasmodial activity of the crude extracts and the pure compounds **2**, **3** and **4** is commensurated with the ethnopharmacological uses of this plant and thus substantiated its usage in BTM for the aforementioned disorders. However, it may be premature to claim that the plant extract formulated and administrated according to traditional protocols would be safe and effective for treating humans. Therefore, *in vivo* animal model of malaria using methods of traditional preparation and administration would more confidently support the use of the plants in treating humans. As resistance to the front line antimalarial drug, artesunate, appears to be emerging (Dondorp et al., 2009), the above *in vivo* experiments on crude extracts and also on compound **2, 3** and **4** which showed significant antiplasmodial activity against antifolate sensitive and multidrug resistant strains are essential and will be possibly carried out in future.

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