European Journal of Biotechnology and Bioscience
ISSN: 2321-9122
Impact Factor: RJIF 5.44
www.biosciencejournals.com
Volume 5; Issue 5; September 2017; Page No. 47-48

Anti-leshmanial constituents from *Corydalis govaniana* Wall.

[1] Ram Lal Shrestha, [*2] Achyut Adhikari
[1] Department of Chemistry, Amrit Campus, Tribhuvan University, Kathmandu, Nepal
[3] Central Department of Chemistry, Tribhuvan University, Kathmandu, Nepal

Abstract
Four tetrahydro-protoberberine type alkaloid; govaniadine (1), caseadine (2), stylopine (3), and fagarine I (4) were isolated from *Corydalis govaniana* Wall. Their structure were deduced using different mass and NMR techniques. Compounds 1-4 were subjected to *in vitro* anti-leshmanial (*L. major*) activity. Compound 1 showed significant activity (IC_{50} = 27.0± 0.2 µg/mL) against *L. major*.

Keywords: *corydalis*, govaniadine, anti-leshmanial, *l. major*

Introduction
Corydalis govaniana Wall. is an important herb and has been used to cure scrofula, syphilis, diarrhea and dysentery. Also, secondary metabolite of these plant has been showing inhibitory effect against hepatitis virus, amoeba, tumors, liver cancer, as well as acesodyne and sedative, improved immunological function, hepatocirrhosis, ascites, etc [1]. The excellent bioactivity profile and ethno-botanical uses of these plants attract us to isolate fully characterized pure compounds and for bioassay screening of these compounds.
Leishmaniasis is a disease caused by the protozoan parasite, such as *Leishmania infantum*, *L. donovani*, *L. maxicana*, *L. chagasi*, *L. amazonesis*, *L. major*, *L. aethiopica*, *L. brasiliensis*, *L. tropica*, etc. Leishmaniasis is wide spread all over the tropical and sub-tropical regions of Africa, Southern Europe, South and Central America, Asian and Mediterranean regions [2].
Some synthetic drugs are used in the chemotherapy of leishmaniasis, many of which are not so effective or toxic to the host. Some drugs such as stibamine, megulamine antimoniate, sodium stibogluconate, etc. cause harsh undesirable effects. Some of the drugs which are in current use such as amphotericin B and pantamidine are toxic and nonresponsive. Failure of treatment is also common [3, 4]. There is an urgent need to develop effective and nontoxic drugs in order to combat the painful disease.

Materials and Methods
Plant Collection and Extraction
The whole plant of *C. govaniana* was collected from Langtang, Rasuwa, Nepal, and identified by Mr. Sanjiv Kumar Rai, Taxonomist, Department of Plant Resources, Thapathali, Kathmandu, Nepal. A voucher specimen, CG-207, has been deposited in Central Department of Botany, Tribhuvan University, Kirtipur, Kathmandu, Nepal.
Air-dried whole plant powder was soaked and extracted with methanol. After evaporation under reduced pressures, the residue was stirred with 7% citric acid for five hours and filtered and neutralized with ammonia solution and extracted with chloroform. The chloroform extract was subjected to column chromatography over silica-gel column by using acetone/hexanes with a few drops of diethylamine with increasing polarity, which afforded the compounds 1-4.

Antileishmanial Activity Assay
Leishmania promastigotes were grown in bulk early in modified NNN biphasic medium by using normal physiological saline. *Leshmania* parasite promastigotes were cultured with RPMI 1640 medium, supplemented with 10% heat inactivated foetal bovine serum (FBS). Parasites at log phase were centrifuged at 2000 rpm for 10 minutes, and washed three times with saline at same speed and time. Parasites were diluted with fresh culture medium to a final density of 1×10^6 cells/mL. The compounds to be checked were dissolved to a final concentration of 1.0 mg in 0.1 mL of PBS (Phosphate Buffered Saline, pH 7.4 containing 0.5% MeOH, 0.5% DMSO). In a 96-well micro titer plate, 180 µL of medium was added in first row and 100 µL of medium was added in other wells. 20 µL of the experimental compound was added in medium and serially diluted. Then, 100 µL of parasite cultures was added in all wells. Two rows were left for negative and positive controls. Negative control received medium, while the positive control contained varying concentrations of standard antileishmanial compound e.g., amphotericin B and pentamidine. The plate was incubated between 24-26 ºC for 72 hours. Then the culture was examined microscopically and parasites were counted on an improved neubauer counting chamber and IC_{50} values of compounds possessing antileishmanial activity were calculated by software Ezfit 5.03, Perella Scientific. All assays were run in duplicate All assays were run in duplicate [5, 6]

Results and Discussion
Details of structure elucidation of compounds 1 and 2 has already published in our previous paper [1]. Structure of compounds 3 and 4 were deduced from different mass and

NMR techniques. All the physical and spectral data of compounds 3 and 4 were found to be similar with reported compounds from same genus [7, 8].

Crude extract of *Corydalis govaniana* showed significant antileishmanial activity, and pure compounds from same plants also showed significant to good activity. Compound 1 showed significant activity (IC_{50} = 27.0± 0.2 µg/mL) against *L. major*. In our previous study compound **1** showed potent antileishmanial activity (IC_{50} = 0.18 µg/mL) against *Leishmania amazonensis* as compared to standard drug amphotericin B (IC_{50} = 0.29 µg/mL) [9]. Activity of compound 1 against different species of *Leishmania* indicated that compound 1 will be lead for antileishmanial drug discovery.

Table 1: Antileishmanial activities of extract and pure compounds.

Name of Compounds	IC_{50} (µg/mL± S.D) of *L. major*	Remarks	IC_{50} µg/mL± S.D of Standard Drugs of both Strains
Govaniadine (1)	27.0±2	Significant	
Casieadine (2)	41.58±0.09	Good	
Stylopine (3)	>100	No Activity	Pentamidine 5.09±0.09
Fagarine I (4)	81.91±0.082	Low	Amphotericin B 0.29±0.05
Crude Extract	26.24±0.05	Significant	

S.E.M. = Standard Error of Mean at n=3

[a] Amphotericin B, and Pentamidine were used as standard

Acknowledgements

We thank Prof. Dr. M. Iqbal Choudhary, International Center for Chemical and Biological Sciences, University of Karachi, Karachi, Pakistan for providing laboratory facility. We thank Dr. Bishnu P. Marasini for critically reading this article.

References

1. Shrestha RL, Adhikari A, Marasini BP, Jha RN, Choudhary MI. Novel inhibitors of urease from *Corydalis govaniana* Wall. Phytochem Lett. 2013; 6: 228-231.
2. Chakravarty J, Sundar S. Drug Resistance in Leishmaniasis, J Glob Infect Dis. 2010; 2(2):167-176.
3. Ram VJ, Nath MP. Progress in chemotherapy of Leishmaniasis. Curr Med Chem. 1996; 3:303-316.
4. Kam T, Sim K, Kayono T, Toyoshima M, Hayashi M, Komiyama K. Cytotoxic and leishmanicidal aminoglycosteroids and aminosteroids from *Holarrhena curtisii*. J Nat Prod. 1998; 61:1332-1336.
5. Habtemariam S. In vitro antileishmanial effects of antibacterial diterpenes from two *Ethiopian Premna* species: *P. schimperi* and *P. oligotricha*. BMC Pharmacol. 2003; 3:1-6.
6. Sousa MC, Varandas R, Santos RC, Santos-Rosa M, Alves V, Salvador JAR. Antileishmanial activity of semisynthetic lupane triterpenoids betulin and betulinic acid derivatives: synergistic effects with miltefosine, PLoS ONE. 2014; 9(3):e89939.
7. Takao N, Iwasa K, Kamigauchi M, Sugiura M. Studies on the alkaloids of papaveraceous plants. XXV. Biosynthesis of the a of *Corydalis incisa* PERS. and *Chelidonium majus* L. incorporations of tetrahydroprotoberberines, N-Methosalts of tetrahydroprotoberberines, and Protopine. Chem Pharm Bull. 1976; 24:2859-2868.
8. Li HL, Zhang WD, Han T, Zhang C, Liu RH, Chen HS. Tetrahydroprotoberberine alkaloids from *Corydalis saxicola*. Chem Nat Compd. 2007; 43:173-175.
9. Callejon DR, Riul TB, Feitosa LG, Guaratini T, Silva DB, Adhikari A, *et al.* Leishmanicidal evaluation of tetrahydroprotoberberine and spirocyclic erythrina-alkaloids. Molecules. 2014; 19(5):5692-5703.