Phytochemical analysis, bioassays and the identification of drug lead compounds from seven Bhutanese medicinal plants

Phurpa Wangchuk

Abstract

The Bhutanese traditional medicine (BTM) has been integrated with biomedicine since 1967 and serves as one of the important health care delivery systems in Bhutan. Therefore, the need to improve its quality through scientific studies have become paramount. In our efforts to address this important issue, we authenticated the botanical names of low altitude medicinal plants, determined the major classes of phytochemicals of 25 selected species, evaluated the bioactivities of the crude extracts of seven of them, and finally isolated the phytochemicals from six of these plants. We have established the antimalarial, anti-inflammatory, antimicrobial, and cytotoxicity activities of these phytochemicals and also identified five novel drug lead compounds. In our ethnobotanical study; we resolved the traditional and taxonomical discrepancies for Bhutanese medicinal plants, assessed the current BTM formulations, and carried out the field survey for the low altitude medicinal plants (LAMP), which is described in Chapter 2. Out of 113 LAMP, 92 species are used in the current multiingredient formulations produced by Manjong Sorig Pharmaceuticals (MSP) and 28 of them are currently imported from India. Out of these 28 species, 16 of them are found growing abundantly in Bhutan and most of them have not been studied for their phytochemicals and pharmacological properties. In this study, we have selected 25 of these plants and assessed them for their major classes of phytochemicals which we
have described in Chapter 3. Of these, the crude extracts of seven selected plants 
(*Corydalis crispa*, *C. dubia*, *Ajania nubegina*, *Meconopsis simplicifolia*, 
*Pleurospermum amabile*, *Aconitum laciniatum* and *Codonopsis bhutanica*) were 
investigated for their biological activities and we found that five of them possessed 
significant antiplasmodial activities, one species showed moderate cytotoxicity, two 
species exhibited moderate anti-*Trypanosoma brucei rhodesiense* activity, six species 
exhibited significant to mild TNF-α inhibitory activities, and all seven plants 
exhibited mild antimicrobial activity. The highest antiplasmodial activity was 
exhibited by the chloroform extract of *M. simplicifolia* with an IC₅₀ value of 0.40 
µg/mL against the TM4/8.2 strain without cytotoxicity. The chloroform extract of *C. 
crispa* showed moderate cytotoxicity with an IC₅₀ value of 12.5 µg/mL and the 
highest antiinflammatory activities. The chloroform extracts of *P. amabile* and also *C. 
crispa* exhibited moderate anti-*Trypanosoma brucei rhodesiense* activity with IC₅₀ 
values of 16.1 and 4.6 µg/mL, respectively. The dichloromethane extract of *P. 
amabile* showed the highest antibacterial activity with a MIC value of 312 µg/mL or a 
MIZ value of 14 mm against *B. subtilis*. Guided by these preliminary bioactivity 
results, we have carried out the isolation of the phytochemicals from six of the seven 
aforementioned plants.

A total of 43 compounds were isolated from these plants. Of these, 25 were alkaloids, 
nine were terpenoids, six were furanocoumarins, two were flavones and one was a 
glycosylated flavone. From *C. crispa* (described in Chapter 4), nine isoquinoline 
alkaloids 1-9 were isolated and protopine (1) was identified as the major alkaloid 
component. The characterization of compounds 2 and 3 with complete NMR data 
analysis was achieved by us for the first time. Similarly, from *C. dubia* (described in
Chapter 5), another group of isoquinoline alkaloids 10-15 were isolated along with the alkaloids 1 and 9. Alkaloid 15 was isolated as a new natural product which we named as dubiamine after the plant’s name (C. dubia) and its structure was confirmed from its single crystal X-ray structural analysis. From A. nubigena (Chapter 6), nine compounds, 19-26 belonging to terpenoids, flavonoids and glycosylated flavones were isolated from the essential oil and crude methanol extract. While luteolin (25) was the major constituent of the MeOH extract, (3R,6R)-linalool oxide acetate (19) was the major constituent (75.8 %) of the essential oil. Out of 53 constituent peaks detected by GC/GC-MS, 45 of them were identified with chamazulene (20) as a new subchemotype of the genus Ajania. From the MeOH extract of M. simplicifolia, one new protoberberine alkaloid (27), with protopine (1) as the major alkaloid, and four benzophenanthridine type alkaloids (29-32) were isolated. The new alkaloid was named as simplicifolianine after its plant species, M. simplicifolia. The GC/GC-MS identified 37 constituents from its essential oil with 14 of them (38% of total oil) being fatty acid esters and dimethyl 1,4-benzenedicarboxylate (29.2%) as the major constituent. From P. amabile, 10 known compounds were isolated for the first time. Its essential oil yielded terpenoids (37-40) with (E)-isomyristicin (37) as the major constituent, and the crude MeOH extract yielded furanocoumarins (41-46) with bergapten as the major one. Through GC/GC-MS analysis, we have also identified 52 compounds from the essential oil component of this plant. From A. laciniatum, five known lycoctonine-type C₁₉-diterpenoid alkaloids (54-58), with pseudaconitine (54) as the major compound, were isolated.

Following the same bioassay methods conducted for the crude extracts of the selected seven plants, we tested 32 of the total of 43 compounds isolated from the six plants
investigated. Of these, 18 compounds showed interesting biological activities. Among these 18 active compounds, protopine (1), cheilanthifoline (11), scoulerine (14), luteolin-7-O-β-D-glucopyranoside (26) and simplicifolianine (27) exhibited highly significant antiplasmodial activities against chloroquine sensitive and multidrug resistant strains of *P. falciparum* and were identified as antimalarial drug lead compounds. Scoulerine (14) significantly inhibited acetylcholinesterase with a minimum inhibitory requirement of 0.0015 nmol which is almost twofold better than that of galanthamine (0.003 nmol), the currently used standard drug to treat Alzheimer disease. Only protopine showed anti-inflammatory activity against TNF-α with a statistically significant *p*-value of *p*<0.05. Apart from compounds 25 and 26, which exhibited mild cytotoxicity, the other compounds were not cytotoxic.

In general, the biological activities of the crude extracts of the seven selected medicinal plants and the 18 pure compounds that we have studied were in alignment with their ethnopharmacological uses. Thus, our studies have validated the use of these plants in BTM either individually or in combination with other medicinal ingredients to treat diseases (especially malaria) as described in the pharmacopoeia and the formulary compendium of BTM.