

PHYTOCHEMISTRY AND BARCODING OF MEDICINALLY IMPORTANT ORCHIDS OF BANGLADESH

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ABSTRACT

One year Post Doctoral Research entitled "Phytochemistry and barcoding of medicinally important orchids of Bangladesh" has been carried out at the University of Aberdeen, Aberdeen, UK from March 2010 to January 2011 under IDB (Islamic Development Bank) Post Doctoral Merit Scholarship. The major aims of the study are to exploring the efficacy of medicinally important orchids and find out the bioactive compounds as well as to sequence the DNA barcoding for proper and authentic identification of the species.

The main three themes of the research work was carried out in three different laboratory under three supervisors: 1. Taxonomy and Phylogeny with Dr. Chris Wilcock, Senior Lecturer in Plant and Soil Science based in Herbarium, 2. DNA sequencing and Barcoding with Dr. Adam Price, Reader of Plant Molecular Genetics based in the Molecular Biology laboratory, and 3. Professor Marcel Jaspars of Chemistry based in Marine Biodiscovery Centre, Department of Chemistry, University of Aberdeen, Aberdeen, UK.

22 medicinally important orchid species used by the tribal people of Bangladesh was collected from different parts of Kaptai Reserve Forest, Rangamati, Bangladesh to study the DNA Barcoding. All the species was identified and confirmed with the help of existing identified herbarium specimen and consulting relevant literature. Then silica gel dried orchid species were ground and extracted using DNA extraction kit. All orchid species were then run in the PCR with two different barcoding primers, viz.: Mat K gene and rbcL gene. After purifying with Purification kits, the samples were sequenced with sequencer. Nanodrop and gel electrophoresis were used to measure the concentration and presence of DNA including the purity percentage. Three different softwares viz.: 1. Sequencher 4.10.1, 2. Bioedit Sequence Alignment Editor, and 3. Mega 4.1 were used to refine raw sequences into single reliable sequence for phylogenetic analysis. After completing all the steps involved in DNA barcoding, finally 21 medicinal orchid species have been sequenced with rbcL gene and 16 species have been sequenced with Mat K gene and will be submitted to gene bank. Most of the sequenced genes (18 in rbcL and 7 in Mat K) are new to the gene bank and in case of existing genes, they are very similar with our sequenced gene indicating the accuracy of our research findings. Phylogenetic tree of both genes are confirming the proper positioning in the phylogenetic classification. Moreover, barcoding genes ensure proper identification of the medicinally important orchid species in the present study and will be the useful aid to establishment of IPR (Intellectual Property Right) in future.

Among all medicinal orchids found in Bangladesh, eight orchid species were selected based on common distribution and important uses, mainly widely distributed and used for critical diseases like cancer to study the phytochemistry. Orchid sample (parts used by the tribal people of Bangladesh, eg. leaves, stem, bulb) was ground and extracted with methanol and dichloromethane (DCM) and then fractionated with butanol, hexane, DCM and methanol following Kupchan Partition Scheme. All fractions of the studied orchid species were tested for antimicrobial and NF κ B (Nuclear Factor kappa B, ideal test for anti-inflammatory and anti-cancer). In the same time, NMR (Nuclear Magnetic Resonance) data for all fractions were obtained from NMR Varian 400 and LC-MS (Liquid Chromatography-Mass Spectrum) data was obtained for all crude samples. No anti-bacterial activities were observed for two bacterial strains viz.: *E. coli* and *S. aureus* in all fractions of the orchid samples. But 18 fractions (out of 32 fractions) of eight orchid species showed remarkable inhibition in NF κ B test indicating potentially valuable bioactivity. From 18 NF κ B active fractions, two fractions of two different species, *Aerides odorata* and *Cymbidium aloifolium* were selected for further investigation. Then HPLC (High Performance Liquid Chromatography) was performed to isolate the compound and again all compound fractions were tested for NF κ B. After having five HPLC bioactive fractions of both species, again HPLC has been performed to isolate the pure compound and elucidating the structure of the compound. Final isolation of pure compound and its activity is waiting to be tested shortly but may not be completed due to time constraint. Rest of the 16 active fractions are also important to be explored which is really exciting if any kind of further support is offered in near future. Moreover, phytochemical screening to assess the presence of proteins, carbohydrates, saponins, alkaloids, tanins, terpenoids, glycosides and steroids was performed in all eight orchid species. Anti-oxidant activities of all medicinal orchid species were determined to explore their efficacy.

During the research work, a good number of seminars and conferences were participated in where research work was presented. One of the articles was presented in the Flora Malesiana Symposium in Singapore and three articles were presented in Plant and Soil Science, and Chemistry Department, University of Aberdeen, UK. Three other scientific meetings and conferences held in Dundee, IMS (Institute of Medical Science) and Chemistry Department, University of Aberdeen, UK were attended as part of the post-doctoral research activities. Two articles of previous work have been completed and submitted to the good impact factor journals and another five articles on the current work hope to be produced shortly.

Considering all aspects of research including literature review, laboratory work, attending seminar and conference and also producing research article, done in the one year period of time as part of post-doctoral research was very satisfactory, but few works still to be done to complete a very good and excellent research. Furthermore, research as continuation with present work is very important for exploring anti-cancer medicine from my sample (orchids) which is now matter of few more months' time and will be the milestone for modern medicine. It is true that even initially we did not realize that our sample would be so precious and valuable what we are realizing now after getting very interesting result. If we can discover the chemical compound effective to treat cancer, IDB also will be the proud partner of this great achievement and we are expecting to reach our goal soon.

ARTICLES

Citation of the two articles expecting to be produced from previous work

1. Huda MK and Wilcock CC. 2010. Colonisation and diversity of epiphytic orchids on trees in disturbed and undisturbed forests in the Asian tropics. *The Gardens Bulletin, Singapore*. Article submitted.
2. Huda MK and Wilcock CC. 2011. Post pollination effects and breeding system of tropical orchids. *Plant Biology*. Will be submitted soon.

Citation of the three articles expecting to be produced on present work

3. Huda MK, Price AH and Wilcock CC. 2011. DNA Barcoding of some important medicinal orchids of the Asian tropics. *Data ready and to be prepared soon*.
4. Huda MK, Jaspars M, Tabudravu JN, Price, AH and Wilcock CC. 2011. Phytochemical screening of eight medicinally important orchid species. *Data ready and to be prepared soon*.
5. Huda MK, Mark S, Tabudravu JN, Iain D, Jaspars M, Price, AH and Wilcock CC. 2011. NFkB inhibition, antibacterial and antioxidant activities of some medicinal orchid species. *Data ready and to be prepared soon*.

Citation of the two articles expecting to be produced on the findings of on going experiment

6. Huda MK, Mark S, Tabudravu JN, Jaspars M, Price, AH and Wilcock CC. 2011. Inhibition of TNF α -induced activation of nuclear factor kB by orchid (*Aerides odorata*) derivatives ?? Expected to be prepared after isolating and elucidating the structure and final testing
7. Huda MK, Mark S, Tabudravu JN, Jaspars M, Price, AH and Wilcock CC. 2011. The inhibition of TNF α -induced NFkB activation by orchid (*Cymbidium aloifolium*) ??. Expected to be prepared after isolating and elucidating the structure and final testing

ORAL PRESENTATION

Two articles were presented in the international arena:

1. Huda MK and Wilcock CC. 2010. Colonisation and diversity of epiphytic orchids on trees in disturbed and undisturbed forests in the Asian tropics. Presented at the 8th Flora Malesiana Symposium, held on 23rd – 27th August 2010, Singapore Botanic Gardens, Singapore.
(IDB funded to attend this symposium)
2. Huda MK. 2010. Bioactivity and phytochemical screening of medicinal orchids of Bangladesh. Presented at MBC Mini Symposium , held in 9 December 2010, Department of Chemistry, University of Aberdeen, UK

Three talks were presented in three different group meetings

3. Research findings entitled “Phytochemical screening of medicinal orchids of Bangladesh” was presented in Chemistry Department, University of Aberdeen, UK
4. Research findings entitled “DNA Barcoding of medicinal orchids of Bangladesh” was presented in Department of Plant and Soil Science, University of Aberdeen, UK.
5. Research findings entitled “Bioactivity and phytochemistry of medicinally important orchids of Bangladesh” was presented in Department of Plant and Soil Science, University of Aberdeen, UK.