Research On Bioactive Molecules: Achievements And The Way Forward

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Abstract: Research on bioactive molecules is one of the thrust areas of research at the International Medical University (IMU). The bioactive molecules that have attracted the interest of IMU researchers include tocotrienol, astaxanthin, zingerone, apigenin, carrageenan and phycocyanin. There are also projects which focus on the screening of extracts from local plants such as *Elephantopus mollis*, *Morinda citrifolia*, *Pereskia bleo*, *Euphorbia hirta*, *Zinger officinale*, *Mangifera indica* and *Nephelium lappaceum* and *algae* such as *Spirulina* and *Gracilaria*. Characterisation of the toxin from *Bacillus thuringiensis* is another area of active research at IMU. The compounds and extracts from the various organisms are screened for anticancer, antioxidative, antiviral and immuno-modulating activity. There are also studies on the production of recombinant molecules, especially monoclonal antibodies for the detection of house dust mites, *Salmonella typhi* and *Candida*. The pool of faculty with diverse expertise and the active collaboration with public universities and institutions have enhanced the progress of bioactive research at IMU. With the current postgraduate and Bachelor of Medical Science (B. MSc.) programme and the introduction of new programmes in health sciences, there are good opportunities for training of students in the research on bioactive molecules. The future research direction should focus on the mechanisms of action of the bioactive molecules using new approaches such as 'omic' technologies and *in silico* modelling.

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Introduction

Bioactive molecules are substances that have an effect on the cellular function of an organism or living tissue. The bioactivity of such molecules may include both beneficial and adverse effects on the living cell. The bioactive molecules may be derived from natural (biological) resources, recombinant DNA technology or synthetic chemicals. Bioactive molecules from biological resources are a potential source of pharmaceuticals and nutraceuticals. The rich biodiversity of Malaysia attracts much interest in the exploration of its bioresources for new bioactive molecules, in the attempts of discovering new drugs. In addition, food bioactives such as phenolics, carotenoids, alkaloids and terpenoids are the potential chemopreventive agents against cancer[1]. In another line of development, with the advent of biotechnology, the production of recombinant bioactive molecules has attracted a lot of interest. Such recombinant molecules have great potential for commercialisation as they are useful in diagnostic tools. In addition, investigations on the mechanisms of action of toxins and poisons in causing harmful effects on cells are another important area of research in bioactives. In spite of their adverse effects, some of the biotoxins can serve as useful tools in neurological research[2].

The emphasis by the Ministry of Science, Technology and Innovations (MOSTI) in the research on bioactive molecules is reflected in the large number of projects in this are supported by the Science Fund. Furthermore, the government has also established a Centre of Excellence for Pharmaceuticals and Nutraceuticals at the Biovalley in Dengkil to promote research on bioactive molecules. A Strategic Planning Workshop was held on 27 November 2004 to chart the five-year (2005 – 2009) research agenda for IMU. Research on bioactive molecules is one of the areas identified to be given special emphasis. This area has received immense interest among IMU researchers and has shown encouraging progress.

Scope of Research on Bioactive Molecules

The bioactive molecules that have captured the interest of IMU researchers include tocotrienol, astaxanthin, zingerone, apigenin, carrageenan and phycocyanin. Most of the bioactive molecules are natural products from biological resources such as palm oil, ginger, bacteria, algae and plants (Table 1). The bioactivities of interest include anticancer, anti-oxidative, antiviral and immuno-modulating effect.

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There are also several studies on biotoxins and poisonous chemicals, especially pesticides. For instance, biochemical characterisation of mosquitocidal proteins from *Bacillus thurigiensis* is an active area of research[3]. There is also interest in using algae and animal cells for genotoxicity testing of pesticides and textile dyes. In addition, there are studies on neurotoxic effect of the pesticide chloropyrifos on the pyramidal cells of the hippocampus in mice. The combined effect of swim stress and dermal application of chlorpyrifos was also tested.

In addition to natural bioactive molecules, there are several studies on the production recombinant molecules. For instance, there is a project on the production of monoclonal antibodies and recombinant allergens for the detection and diagnosis of house dust mites. There was another project on the development of immunoassays for the detection of Candida antigens in systemic candidiasis. There are several other projects on recombinant proteins with the aim of contributing to the development of diagnostic kits, which have great potential for commercialisation. There is also a project on recombinant proteins such as phytase and Dsn1p from yeasts, which are useful as value-added biopharmaceuticals for industrial application.

Apart from pure compounds, there are many projects which focus on the bioactivity of extracts from various plants and microorganisms. For instance, the extracts from microalgae were screened for bioactivity on nasopharyngeal carcinoma (NPC) cells and Epstein-Barr virus (EPV). Using real-time PCR technique, it was found that certain fractions of the extracts can inhibit the release of EBV from the host cells. In another study, extracts from seaweeds were screened for antiparasitic activity against *Acanthamoeba*. Extracts from plants such *Gingko biloba*, *Pereskia bleo*, *Centella asiatica*, *Euphorbia hirta*, *Kaempferia galangal* and *Morinda citrifolia* were screened for a wide range of bioactivities including antioxidative, anticancer, haemostatic and antimutagenic effect. There is also an ongoing project which aims to explore new natural resources of antioxidants from Malaysian plants such as *Nephelium lappaceum* (rambutan) and *Syzygium maingayi* (jambu merah). A Malaysian patent for the antioxidant activity of the ethanolic extract from the rind of the rambutan fruit has been filed [PI20064567(2006)].

Funding for Research on Bioactive Molecules

Generous funding has been received through the IMU Internal Grants to support the research in bioactive molecules. In 2007, 29 of the 87 approved projects (33%) by the IMU Research Committee (IMU-RC) and IMU Ethics Committee (IMU-EC) were on bioactive molecules.4

In 2004, IMU researchers in collaboration with public universities and research institutions have been successful in obtaining grants through the government's Intensification of Research in Priority Areas (IRPA) funding mechanism[4]. The project on the bioactivity of algae extracts against nasopharyngeal carcinoma (NPC) and Epstein-Barr virus (EBV) was part of a top-down project funded under this mechanism. In 2006, IMU researchers have also successfully obtained external funding through the ScienceFund under the ninth Malaysian Plan for the research on bioactive molecules. One project was on the "Use of algae for genotoxicity testing of the marine ecosystems" while the second one was on "Nutraceutical development from local plant extracts of *Nephelium lappaceum* (rambutan) and *Syzygium maingayi* (jambu merah) with significantly high antioxidant activity". In 2007, a project on production of monoclonal antibodies and recombinant allergens for detection of house dust mites was successful in obtaining funding from the Science Fund. The research on bioactives is not only supported by internal and government funding but also other external sources. For instance, the project on the cytotoxic effect of parasporal inclusions of *Bacillus thurigiensis* on leukaemic cells was funded by the Dr Ranjeet Bhagwan Singh Grant awarded to Dr Vishna Devi Nadarajah. There was also project on the "Use of algae for genotoxicity testing of textile dyes" funded by

the Gwangju Institute of Science and Technology (GIST) in Korea.

Strength and Opportunities in Bioactive Research

The strength of IMU in bioactive research lies in its multidisciplinary faculty, with expertise ranging from microbiology, molecular biology, physiology, chemistry, biochemistry to pathology. The joint effort of a multi-disciplinary team helps to ensure the success in this area of research.

The availability of internal grants is an important factor that has enhanced the development of bioactive research at IMU. The internal grants help to fund the projects for the undergraduate (B. Med. Sci. and B. Pharm.) and postgraduate (M. Sc. and Ph. D.) programmes. This offers a good opportunity for the training of students and to promote interest in bioactive research. It is anticipated that more students will enrol in projects on bioactive molecules with the introduction of new programmes on Bachelor of Medical Biotechnolgy (B. Med. Biotech.), Bachelor of Biomedical Science (B. BMed. Sci.) and Nutrition and Dietetics.

The active collaboration with other universities and research institutions is another factor that has contributed to the development of bioactive research at IMU. Our collaborators include fellow researchers from the University of Malaya, Universiti Putra Malaysia, Universiti Sains Malaysia, Monash University, Universiti Teknologi Mara, Malaysian Palm Oil Board (MPOB) and Institute of Medical Research (IMR). The partnership is particularly important in the application for external funding to drive the research on bioactive molecules. In addition, it allows the sharing of high-end equipment such as RT-PCR, microarray, 2D-gel electrophoresis and electron microscope.

There is good infrastructure at IMU to support the research on bioactive molecules. There are well-equipped facilities such as flow-cytometry, ultracentrifuges, thermocyclers, freeze dryers and HPLC. There are also good cell culture facilities for *in vitro* testing of bioactive compounds. In addition, the animal holding facility allows *in vivo* studies to be carried out. Thanks to the support from the Management, there are plans to further upgrade the research laboratory with more space and better facilities.

Future Research Directions

Most of the projects on bioactive molecules are still on the screening of crude extracts. Some have used standardised extracts while others have started testing the bioactivity of fractions from solvent extracts. Elucidation of the structure of the active principles should be a priority area for further studies, if the extracts did show promising activity.

A potential area for further studies is the use of "-omic" technologies to unravel the mechanism of action of bioactive compounds. The "-omic" technologies based on the technology platform of genomics, proteomics and metabolomics can be applied to relate the complex effects of the bioactives in the form of gene/protein expression profiles[5]. For instance, microarray analysis will give insights into the differential gene expression when the cells are exposed to the bioactive molecules.

With the advent of bioinformatics, it is also time for IMU to venture into *in silico* modelling in the research on bioactive molecules. Such an approach is the current trend in drug discovery whereby the bioactivity of natural products is manipulated through computer simulation[7]. Through combinatorial chemistry, a large number of different but structurally related molecules can be synthesised or analysed by computer simulation.

Most of the projects on bioactive molecules at IMU focus on terrestrial organisms. Perhaps it is time that we should explore the potential of marine organisms as a source of bioactive compounds. The marine ecosystem holds a rich biodiversity with vast potential for biotechnological exploitation that is yet to be fully explored. A wide range of bioactive compounds have been discovered from marine organisms ranging from bacteria, corals, sponges to seaweeds[8]. It is an exciting and challenging task indeed to venture into the search for bioactive compounds from the sea!

Concluding remarks

The research on bioactive molecules at IMU has been progressing well. This is due to the keen interest among the faculty members. The diverse expertise of the researchers is the strength of IMU in bioactive research. The internal funding available and the need for the training of undergraduates and postgraduates are some of the key factors that have enhanced the development of research in this area. It is time that IMU ventures into new areas using approaches such as "-omic" technologies and in silico modelling to understand the mechanisms of action of bioactive compounds. It is worthwhile to explore new biological resources, especially marine organisms as a source of bioactive molecules. This may lead us closer in translation of our aspirations to discover new drugs to reality.

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Table 1: Research on bioactive molecules at the International Medical University[4]

BIOACTIVE MOLECULES	SOURCE	BIOACTIVITY / APPLICATION
Tocotrienol	Palm oil	Immuno-modulation Adjuvant in developing cancer vaccines
Astaxanthin	Algae (*Haematococcus*) & Synthetic	Anticancer and antioxidative activity
Zingerone [6]-Gingerol	Ginger	Anticancer and apoptotic activity
Tannins	*Mimosa pudica*	Snake venom neutralization activity
Coumarins	Synthetic	Antiproliferative activity on breast cancer cell lines
Phycocyanin	Algae (*Spirulina*)	Antiproliferative activity on breast cancer cell lines
Carrageenan	Algae (*Seaweeds*)	Protective effect against UVR-induced toxicity and mutagenicity
Toxin / proteins	*Bacillus Thurigiensis*	Anticancer effect on leukaemic cells
Apigenin	Synthetic	Effect on cyclosporine A induced renal damage
Quercetin	Synthetic	Effect on diabetic vascular tissue
Textile dyes	Synthetic	Mutagenic and genotoxic effects on algae and animal cells
Pesticides	Synthetic	Mutagenic and genotoxic effects on algae and animal cells
Huperazine-A	Synthetic	Effect on aluminium chloride-induced changes in the hippocampus of mice
Monoclonal antibody	Recombinant Proteins	Diagnosis of house mite allergens
Polyclonal and monoclonal antibodies	Recombinant Molecules	Filarial antigen detection assay for *Brugia malayi*
Monoclonal antibodies	Recombinant Proteins	Immunoassay for the detection of *Candida* infection
Proteins	Recombinant Proteins, *Salmonella Typhi*	Diagnosis of typhoid fever

Source : IMU President's Reports (2004, 2005, 2006, 2007)