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Journal of Integrative Medicine (JIM) is a continuation of the *Journal of Chinese Integrative Medicine* (phonetic romanization of the Chinese title used by MEDLINE/PubMed: *Zhong Xi Yi Jie He Xue Bao*) established in 2003. Our international and multi-disciplinary advisory and editorial boards ensure the journal's scientific integrity and international diversity. Our commitment is to make JIM an international publication platform for high-quality papers on complementary and alternative medicine (CAM) and an open forum in which the different professions and international scholarly communities can exchange views, share research and their clinical experience, discuss CAM education, and confer about issues and problems in various disciplines and in CAM as a whole in order to promote integrative medicine. The scope of the journal covers all aspects of integrative medicine, such as acupuncture and traditional Chinese medicine, Ayurvedic medicine, herbal medicine, homeopathy, nutrition, chiropractic, mind-body medicine, Taichi, Qigong, meditation, and any other modalities of CAM.

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Notes from the Editor-in-Chief

Lixing Lao, PhD, MB, Professor and Director, School of Chinese Medicine, the University of Hong Kong, China

The European Congress for Integrative Medicine (ECIM) 2015 Global Summit on Integrative Medicine and Healthcare was held on September 26–27, 2015 in Greater Copenhagen. Participants from all around the world explored the prominent issues that included global healthcare and integrative medicine models, integrated healthcare workforce, education and patient care. The Summit drew world's attention and successfully promoted the Integrative Medicine to the public eye once again.

According to the definition by the Academic Consortium for Integrative Medicine and Health, “integrative medicine and health reaffirms the importance of the relationship between practitioner and patient, focuses on the whole person, is informed by evidence, and makes use of all appropriate therapeutic and lifestyle approaches, healthcare professionals and disciplines to achieve optimal health and healing” (<https://www.imconsortium.org/about/about-us.cfm>). Integrative medicine is described as the art and science of healthcare by Nordic Integrative Medicine. In the United States, Australasia and Europe, the primary medical doctors have treated integrative medicine as another important choice when they deliver healthcare to patients (<http://www.nordicintegrativemedicine.com/what-is-integrative-medicine>).

To introduce the development and current status of integrative medicine, in terms of clinical usage, education and research, in Europe and other Western countries, we will publish articles on this topic presented by the Summit speakers as a series in this and the coming editions of the *Journal of Integrative Medicine*. I hope our readers will find these articles interesting and useful in the field of integrative medicine.

A Summary of the ECIM 2015 Global Summit on Integrative Medicine and Healthcare

Shelley R. Noble-Letort, PhD, Founder and Chairman of Nordic Integrative Medicine, 8th ECIM President

In September 2015, Nordic Integrative Medicine (NIM), in partnership with the European Society of Integrative Medicine (ESIM), convened the 8th European Congress for Integrative Medicine (ECIM) 2015 Global Summit on Integrative Medicine and Healthcare in Greater Copenhagen, Denmark, to explore the prominent issues that healthcare is facing globally and the scientific evidence for or against integrative medicine (IM) that includes conventional medicine (CM), complementary and alternative medicine (CAM), and traditional medicine (TM).

1 Background of the Global Summit

ECIM 2015 Global Summit was inspired by the historical 2009 Summit on Integrative Medicine and

the Health of the Public in Washington D.C. hosted by the Institute of Medicine (IOM) and the Bravewell Collaborative. Global Summit keynote speaker, Dr. Woodson Merrell, MD, Executive Director of the Center for Health and Healing and Chairman of the Department of Integrative Medicine, Mount Sinai Beth Israel Medical Center in New York City, reminded us that the call from 2009 for the need for reconfiguration of healthcare has not changed. He stated that, “Integrative medical care that increases patient satisfaction and decreases the use of costly pharmaceutical and procedure-intensive therapies remains the key to fixing this globally broken system, and yet academic medical centers have only cautiously embraced the integrative healthcare concept. Common perceived hurdles are not only the evidence-basis—

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something increasingly answered by a near-explosion of quality research—but also practical hurdles, especially legal and financial.”

Important points articulated by the 2009 Summit faculty were: (1) The progression of many chronic diseases such as cardiac disease and cancer can be reversed and sometimes even completely healed by making lifestyle modifications. (2) Genetics is not destiny and gene expression can be turned on or off by nutritional choices, levels of social support, stress reduction activities such as meditation, and exercise. (3) Our environment influences our health—the environment outside one’s body rapidly becomes the environment inside the body. (4) Improving our primary care and chronic disease care systems is paramount. (5) The reimbursement system must be changed to encourage healthcare providers to focus on the health outcomes of their patients. (6) Changes in education will fuel changes in practice. (7) Evidence-based medicine is the only acceptable standard. (8) A large national demonstration project that substantiates the clinical and cost-effectiveness of an integrative approach to care is needed.

According to the Bravewell Collaborative, “IM is founded on the premise that health is a state of physical, emotional, mental, social and spiritual wellness, which enables an engaged relationship with life. When health in its fullest sense is the goal of the healthcare system, then it naturally follows that all the influencing factors, not just the physical problems, need to be addressed in the care process.” As Dr. Harvey Fineberg, MD, PhD, former President of IOM and host of the 2009 Summit stated, “One of the most important reasons to have this Summit is to focus attention on healthcare for the whole person.”

2 Participants of the Global Summit

More than 30 nations and 40 universities represented over 100 scientific presentations. Health practitioners, scientists, policy experts, academic and industry leaders and patient advocates from across the globe came together to share their research and experience, and suggested how the principles and practices of IM could help patients, providers and payers. “As the role of governments, as payer, regulator, and market-shaper, is growing, many governments, such as the United States, China, India, South Africa, Mexico and Rwanda, are introducing broad reforms to healthcare systems,” said Global Summit opening keynote speaker, Dr. Victor Dzau, MD, President of the National Academy of Medicine (formerly called IOM), Washington D.C., “There is a need for earlier detection and intervention and so healthcare systems must change and focus on integration, prevention of disease, health and wellness, treating the whole person, and becoming more community-based.” Dr. Dzau emphasized the need for a collective research mission:

(1) assess the effectiveness of integrative, personalized models of care (especially cost-analysis); (2) evaluate efficacy of model components when possible or strategic for funding (*e.g.*, mindfulness approach to change eating behavior); (3) collaborate with basic scientists to develop biological correlates of IM clinical outcomes and explore potential mechanisms of action (in effectiveness and efficacy studies).

A pre-summit policy roundtable report delivered on behalf of Professor Dr. Negoslav P. Ostojic, CEO Executive Director of the United Nations European Center for Peace and Development (UN ECPD), declared a call for strategic policies in support of integrated healthcare that would include a vision for setting up “an IM department in at least one hospital in every European country.” The report continued by stating that, “Political will and support will be essential in that process as hospitals and officials would not change standard protocols and practice without push from the outside and above.” UN ECPD has Diplomatic Status as part of the academic system of the UN with specific tasks and actions for sustainable development, education, and research. The report elaborated on the need for smart strategic planning, management and promotion in the following fields. (1) **Sustainable Development:** Environmental and human health is not just the absence of disease rather it is measured by outside factors as well as inside factors. Because “where we live, and what we do, have, eat, drink matters,” environmental and health agencies need to work together to create immediate solutions. (2) **Education:** Quality-standardized education is crucial to obtain a sufficient number of present and future TM/IM health providers. Most health professionals are not educated about TM/CAM/IM, and statistics suggest that 30% wish to learn relevant basics. There are few education curricula available in most European Union countries and most governing bodies are not in favor of any change. (3) **Shared Knowledge/Databases:** Evidence suggests that there are 1 260 herbal drugs in the national essential drug list in China and yet out of 22 countries (16% of 135 total who include herbal medicines on their respective national essential drug list), the majority of the countries had listed only one to 10 herbal medicines. It takes thousands of years to develop that level of ancient medicinal knowledge and yet we hesitate to use it. (4) **Research:** Research is important but must be balanced. There exists a constant push for research and little use of its results. European researchers already have some evidence/results for the IM approach towards better health & prevention that can be applied to patient care. Dr. Konstantin Cesnulevicius, MD, Medical Science Liaison for Heel GmbH and Professor Stefan Willich, MD, University of Berlin Charité and Founder of ESIM presented the ESIM innovative award “Excellence in Integrative Medicine Research”. Four researchers (China, Sweden and the United States) shared

this international prize, which recognizes innovative and excellent scientific research in the field of IM in cooperation with conventional and complementary medicine (<http://www.nordicintegrativemedicine.com/ecim-2015-excellence-in-integrative-medicine-research-award/>).

For a link to the published academic research abstracts online at the *European Journal of Integrative Medicine* and a list of the prominent keynote speakers, leaders in the field of IM in their respective countries of Australia, Brazil, China, Denmark, Germany, Norway, Portugal, Sweden, Switzerland, the United Kingdom, and the United States, please visit the NIM website (<http://www.nordicintegrativemedicine.com/ecim-2015-keynote-speakers>).

3 Theme of the Global Summit

The theme of the Global Summit was “Exploring the Evidence-Base for Integrated and Sustainable Research, Healthcare and Workforce for Patients” that, for the first time, truly placed the patient at the center of the congress and offered an innovative, solution-driven platform for physicians and healthcare practitioners, researchers and students, politicians and patients, to collectively exchange and discuss research and research findings in the field of IM and collectively work on the vision and science, economics and education for optimal healthcare. There were moderated academic and scientific sessions and open-forum expert panel discussions with the opportunity for delegate feedback that included two sponsored ReThinkBox Sessions: Integrative Oncology and Patient Engagement.

A powerful post-humous video keynote by patient expert, Michael Hay, former Creative Director for IKEA Global Communications, delivered important directives to city mayors and doctors: “In 30 years, 70% of the world’s population will live in cities, so in the future, city mayors will become more important than politicians and the really visionary mayors will be the real leaders in sustainable development. There are so many options for patients

and doctors. By working together and by opening up from both sides, you can create a team, which is going to make life better for both the doctors and the team treating the patient and the patient, who starts feeling less like a patient and more like a human being, who is getting help. Anything that can give hope every time you meet, without distorting reality, is worth so much.”

Key questions that were addressed at the Summit included: Can IM provide high-quality yet cost-effective care for global health? Can IM research offer solutions for global health that are truly sustainable? Can IM improve patient-centered healthcare through an integrated workforce?

Directed by the ECIM 2015 Pre-Summit Invitational Roundtable, a Nordic Policy for Integrative Healthcare is currently being drafted by Roundtable Chairman Professor George Lewith of Southampton University, NIM Chairman Dr. Noble-Letort and the NIM Nordic Council. The Nordic Policy will be translated into all 5 Nordic languages.

4 Nordic Integrative Medicine

The mission of Nordic Integrative Medicine is to “move healthcare into sustainability” by becoming the leading provider of IM and the green healthcare model for Scandinavia and Europe that catalyzes a sustainable future for healthcare and empowers people to create flourishing lives (<http://www.nordicintegrativemedicine.com>).

5 European Society of Integrative Medicine

The aim of the European Society of Integrative Medicine is the advancement of science, research, education and further training, support for medical care and providing advice on policy in the realm of integrative medicine. This includes holding scientific events and conducting dialogue with professional health care and public health associations and institutions (<http://www.european-society-integrative-medicine.org>).



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• Editorial

Integrative medicine, or not integrative medicine: that is the question

Malcolm B. Taw

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ABSTRACT

On September 26–27, 2015, the 8th European Congress for Integrative Medicine convened the Global Summit on Integrative Medicine and Healthcare in Greater Copenhagen and Helsingør, Denmark at the Culture Yard just across from Kronborg Castle, which is home to William Shakespeare's Hamlet.

This article is a summary of the author's presentation about integrative medicine within the Nordic region, driving factors that determine value in healthcare, key tenets of integrative medicine that lead to healthcare cost savings and the potential for a Nordic healthcare renaissance.

Keywords: integrative medicine; Nordic; Shakespeare; Hamlet; Renaissance; healthcare; value; cost-effectiveness

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1 Introduction

On September 26–27, 2015, the 8th European Congress for Integrative Medicine convened the Global Summit on Integrative Medicine and Healthcare in Greater Copenhagen and Helsingør, Denmark with over 30 countries represented. The event was held at the Culture Yard, an architecturally innovative center just across from Kronborg Castle, which is home to William Shakespeare's *Hamlet*.

2 An existential question

“To be, or not to be: that is the question”^[1]—these well-known opening words uttered by Hamlet during his soliloquy when contemplating the very question of life and death are quite apropos to the existence of integrative medicine within the Nordic region and, in particular, the nation of Denmark where it is virtually nil. Ironically, Hamlet, who was a Prince of Denmark, could very well

have applied these words to his home country.

3 Does integrative medicine bring value?

Inherent within the existential question of integrative medicine is the determination of value. If integrative medicine presents value, then this would support the need for its existence; however, a lack thereof, would argue against it.

The core metrics of healthcare that drive such a decision include: 1) health outcomes (both objective, disease-related outcome measures and subjective, patient-centered reported outcomes), 2) effectiveness/efficacy, 3) science, 4) research, 5) safety, 6) quality improvement and 7) cost-effectiveness, which determines appropriate resource allocation and, ultimately, where healthcare dollars should be spent. Victor Dzau, M.D., President of the National Academy of Medicine (formerly Institute of Medicine), had

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emphasized during the summit that value can be defined as quality divided by cost^[2].

4 Healthcare systems and cost

The Organisation for Economic Co-operation and Development (OECD), which currently has 34 member countries including all Nordic nations, published a working paper that evaluated the strengths and weaknesses of each country's healthcare system and assessed the scope for improving value-for-money^[3]. Its conclusion was that there is no healthcare system that performs systematically better in delivering cost-effective healthcare and that all countries have room to improve healthcare spending efficiency. To no surprise, the OECD also found that most countries within the European Union have annual healthcare costs that are rising faster than the economy.

Broadly speaking, there are 2 major healthcare system cost models: 1) revenue generating with a reliance upon market mechanisms in service provision and 2) cost saving with a single source of coverage and provision. Consequently, the former healthcare system tends to be volume-driven and high technology-oriented, which flourishes in a fee-for-service, third-party insurance "open" system model. In contrast, the latter healthcare system is more value-driven, embraces low technology and produces cost savings in a "closed" model such as a single payer system, health maintenance organization, capitated system or accountable care organization. In a cost-saving model, a stronger emphasis is also placed upon prevention and wellness.

5 Integrative medicine is inherently a cost-saving healthcare model

A 6-year economic evaluation of healthcare costs and mortality rates was conducted in the Netherlands, which compared a conventional approach among general practitioners (GPs) versus care provided by GPs who had additional training in complementary and integrative medicine (CIM)^[4]. The main outcome measures were annual healthcare costs accrued from care by GPs, hospitalizations, pharmaceuticals, paramedic care and care from supplementary insurance.

This study demonstrated that Dutch patients who received care from GPs that provided CIM had a 10% reduction in total annual healthcare costs, most of which was attributed to lower hospital care and pharmaceutical costs, and with similar mortality rates when compared to patients who received conventional care.

Herman and colleagues^[5] had done a comprehensive systematic review of economic evaluations of CIM

published from 2001–2010, which yielded 338 economic evaluations with 114 full evaluations. Among the higher-quality studies, nearly 30% demonstrated cost savings as shown by cost-effectiveness, cost-utility and cost-benefit comparisons for CIM therapy versus usual care.

6 Key tenets of integrative medicine that lead to cost reduction

6.1 Holism

In 2009, the Institute of Medicine convened the Summit on Integrative Medicine and the Health of the Public, during which Dr. Ralph Snyderman articulated a shift in healthcare paradigm whereby the 19th–20th centuries focused on 'reductionism' with a resulting emphasis upon the fields of chemistry, physics, physiology and pathology, while the 21st century exhibits 'holism' with a greater significance placed upon the subdisciplines of systems biology, genomics, proteomics, metabolomics and bioinformatics^[6].

This transition in healthcare paradigm was also described by Federoff and Gostin where systems medicine and 'care for the whole person' manifest dynamic interactions among all components of health and disease through integration of multiple networks and connectivity that exists beyond reductionism^[7]. From this, arises a key feature of 'emergent properties'—a phenomenon that exists only among complex whole systems, but not found within their individual parts. Through utilizing the diagnostic and therapeutic power of holism and harnessing the unique quality of emergent properties, the entire system of the human body can be treated in a congruent and unified fashion and, hence, can have important economic implications and cost-saving potential.

6.2 Homeostasis, allostasis and the innate healing response

The human body has an incredible innate healing capacity to maintain homeostasis (physiological parameters essential for maintaining life) in the midst of continual environmental perturbations through adaptive preservation of its internal milieu (*e.g.*, pH, oxygen tension, temperature regulation)^[8]. This occurs via mechanisms of allostasis (achieving stability through change), such as feedback production of various hormones like cortisol or other mediators that modulate the autonomic nervous system, inflammation and immune system.

One of the goals of integrative medicine is to augment and facilitate this inherent healing response through various lifestyle changes (*e.g.*, balanced diet, moderate exercise, stress management, optimal sleep quality) and other therapeutic interventions, such as acupuncture. As described by Li and colleagues^[9], "acupuncture



restores the homeostatic balance by a differential effect of suppressing hyperfunction, stimulating hypofunction, and regulating disturbed function.” Hence, a unique therapeutic attribute of acupuncture is its concurrent treatment of both hyper- and hypofunction, whereas conventional allopathic pharmacotherapy primarily treats either one.

For example, in a patient with irritable bowel syndrome from which one may experience alternating diarrhea and constipation, the conventional Western medical approach is to prescribe medication depending upon the predominant symptom. In contrast, acupuncture can treat both diarrhea and constipation simultaneously via re-regulation of the gastrointestinal physiology.

Through incorporation of various lifestyle changes and low-cost interventions that are inherently holistic and homeostatic to achieve high-quality outcomes and clinical effectiveness, integrative medicine has the potential to reap large healthcare cost savings.

7 The Renaissance

The Renaissance period has certainly left an indelible mark upon history. Within the Nordic region, the royal castle of Kronborg, a strategic coastal fortification that controlled the narrowest portion of the Øresund strait between Denmark and Sweden, was one of the most important castles built during the Renaissance. William Shakespeare, who is widely considered the world’s greatest playwright and author in the English language, had written *Hamlet* during the height of the Renaissance period.

More recently, over the past 15 years, the new Nordic cuisine has brought a resurgence of interest in traditional Scandinavian fare leading to a type of ‘culinary renaissance’ with Copenhagen being home to the best restaurant in the world, *Noma*, for 4 of the last 5 years^[10] and the entire Nordic region becoming a gastronomic haven with a wealth of Michelin star restaurants^[11]. Reasons for this nouvelle cuisine are numerous, but perhaps the most important is the integration of innovation with tradition, the avant garde with the age-old.

Could the Nordic region be poised to experience yet another renaissance, and more specifically a Nordic healthcare renaissance? There seems to be much in place for such a transformation to occur: fresh, natural and organic food sources; lush verdant greens; an abundant clean water supply; a culture where walking and bicycling are the preferred modes of transportation; low pollution; a national healthcare system that can reap the rewards of a cost-saving model and a region where all 5 Nordic nations consistently rank among the top 10 happiest countries in the world^[12]. All that remains is the integration of

healthcare innovation with tradition.

Perhaps, eventually there will no longer be any reason to question the existence and need for integrative medicine in the Nordic region. Integrative medicine will someday be its own *raison d’être*.

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9 Disclosure of competing interests

The author declares that he has no competing interests.

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• Commentary

Integrating traditional Chinese medicine into mainstream healthcare system in Hong Kong, China

—A model of integrative medicine in the HKU-SZ Hospital

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ABSTRACT

The European Congress for Integrative Medicine 2015 Global Summit on Integrative Medicine and Healthcare in Greater Copenhagen has successfully promoted integrative medicine to the public once again. Integrative medicine, which is called the art and science of healthcare by Nordic Integrative Medicine, has been widely used in the world. In Hong Kong, integrated traditional Chinese and Western medicine, which is also known as the Chinese version of integrative medicine, provides a valuable reference for the development of integrative medicine in the world. In this article, we introduce the development of traditional Chinese medicine in Hong Kong and an integrated traditional Chinese and Western medicine model in the University of Hong Kong-Shenzhen Hospital.

Keywords: integrative medicine; traditional Chinese medicine; Hong Kong

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1 Introduction

Hong Kong, which is known as a typical representative of “one country, two political systems”, is also a typical representative of “one region, two medical systems”. The integration of traditional Chinese medicine (TCM) and Western medicine (WM) is the Chinese version of integrative medicine^[1]. Integrated traditional Chinese and Western medicine in Hong Kong provides a valuable reference for the development of integrative medicine in the world. This article will introduce the development of TCM in Hong Kong, and an integrated traditional Chinese and Western medicine model in the University of Hong Kong-Shenzhen (HKU-SZ) Hospital.

2 Development of TCM in Hong Kong

In the folk of Hong Kong, as a treasure of Chinese

culture, TCM had been deeply rooted in the minds of the people in Hong Kong and used in their daily life. For example, one can easily find Chinese medicine pharmacies and clinics in the streets and alleys, which had existed for decades or even hundreds of years in Hong Kong. In addition, almost in every family of Hong Kong, the Chinese Hong Kong people have a habit of stewing soup with Chinese herbal medicine. As a folk medicine, TCM showed a tenacious vitality in the society of Hong Kong. Nevertheless, TCM had not been recognized by the Hong Kong Government as part of the healthcare system.

The popularity of TCM finally caught the government's recognition and attention. In August 1989, the Hong Kong Government appointed the Working Committee on Chinese Medicine to provide advice on how to promote the proper use and good practice of Chinese medicine in Hong Kong. Following the Working Committee's suggestions, the Hong Kong Government appointed

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the Preparatory Committee on Chinese Medicine in April 1995 to make recommendations on the promotion, development and regulation of Chinese medicine in Hong Kong^[2].

After 1997, the Basic Law of the Hong Kong Special Administrative Region indicated the direction of the future development of Chinese medicine. Article 138 of the Basic Law writes that “the Government of the Hong Kong Special Administrative Region shall, on its own, formulate policies to develop Western medicine and traditional Chinese medicine and to improve medical and health services. Community organizations and individuals may provide various medical and health services in accordance with law.” Two years later, the Chinese Medicine Bill was passed by Legislative Council in 1999 and the Chinese Medicine Council of Hong Kong was established in the same year. Since then, Hong Kong has possessed a special law and institution for the management of Chinese medicine^[2].

After more than 10 years, the development of Chinese medicine in Hong Kong has carried all before one. Eighteen Chinese medicine clinics were set up in each district of Hong Kong. Three local universities, namely the University of Hong Kong (HKU), the Chinese University of Hong Kong, and Hong Kong Baptist University, had offered full-time bachelor degree courses on Chinese medicine. Every year, the Research Grant Council and Hospital Medical Research Fund provide research fund for mechanistic studies of Chinese medicine and clinical studies of TCM interventions^[2].

In April 2012, the Chinese Medicine Division of the Department of Health was designated as the “World Health Organization Collaborating Center for Traditional Medicine”. This was a significant support for Hong Kong’s role as an international center of Chinese medicine^[2].

In February 2013, the Chinese Medicine Development Committee was established to give recommendations to the Government on the direction and long-term strategy of the future development of Chinese medicine in Hong Kong^[2], especially on the five areas, including enhancing the professional standards and status of Chinese medicine practitioners; strengthening research and development of Chinese medicine; promoting treatment with integrated traditional Chinese and Western medicine; expanding the role of Chinese medicine practitioners and Chinese medicine in the public healthcare system; and introducing Chinese medicine in-patient services^[3].

In 2014, Article 178 of the Government Policy Report mentioned that “The Chinese medicine sector generally agrees that Hong Kong needs and stands ready to develop a Chinese medicine hospital to provide Chinese medicine in-patient services. This will also help enhance the professional training and standards of Chinese medicine practitioners in Hong Kong^[4].” The Government has

reserved a sizable land for such a hospital. However, the year when the hospital is built and in service is still unknown.

3 Integrated traditional Chinese and Western medicine model in the HKU–SZ Hospital

In 2012, based on the agreement between Shenzhen Government of mainland China and the HKU, a conventional hospital, known as the HKU–SZ Hospital, was built by the Shenzhen Government and managed by the HKU (Figure 1). A department of Chinese medicine was established after the hospital recruited the Chief of Service, Professor Lixing Lao who also serves as the director of the School of Chinese Medicine, HKU in late 2013^[5]. In January 2014, the Department of Chinese Medicine started its out-patient service (Figure 2). There is uniqueness of the Department of Chinese Medicine in this hospital. Its operation of integrated traditional Chinese and Western medicine is different compared with mainland China and Hong Kong. In mainland China, the model of integrated traditional Chinese and Western medicine is the integration of these two medicines into one doctor’s practice; in other words, a patient may receive treatments of TCM and WM given by a single doctor. Contrary to the mainland China, the model of integrated traditional Chinese and Western medicine in the HKU–SZ Hospital is the integration of these two medicines into one patient care, known as patient-centered health care. The patient would receive TCM treatment and WM treatment from the best TCM doctor and WM doctor, respectively (Figure 3).

Since the establishment of the Department of Chinese Medicine, it now consists of 15 doctors, including 3 doctors from the School of Chinese Medicine of the HKU and another 12 doctors from the first-class A-level hospitals in mainland China. The mission and value of the Department of Chinese Medicine of HKU–SZ Hospital



Figure 1 The University of Hong Kong-Shenzhen Hospital panorama



Figure 2 Acupuncture treatment



Figure 3 Collaboration with Western medicine departments

is “to contribute to health and well-being by providing the patient-centered, mind-body wellness-oriented care to every patient through integrated clinical practice with evidence-based, holistic and syndrome differentiation-featured Chinese medicine, herbs, acupuncture and other modalities^[6]”, and there are some characteristics in the daily operation.

1. All medical staff of the Department of Chinese Medicine are trained in Chinese medicine and are familiar with WM. They can use the basic investigational laboratory tests to assist them making diagnosis, but they have no rights to prescribe Western medication.

2. The hospital emphasizes the importance of cooperation of medical team. TCM doctors collaborate with WM departments for patient care and professional Chinese and Western medical cares are provided by Chinese medicine and WM doctors, respectively.

3. All the acupuncture treatment is enforced by clean needle techniques with disposable acupuncture needles, and both raw Chinese herbs and herbal granules are allowed to be prescribed.

The number of patients has increased month by month in the Department of Chinese Medicine since its establishment. The healthcare model of integrated traditional Chinese and Western medicine has been widely recognized by the public in Shenzhen City. In the future, the Department of Chinese Medicine will take measures to promote the development of the integration model in the following 6 aspects.

1. Provide in-patient service for patients after stroke and surgery.
2. Set up *Tuina* special clinics, and provide pediatric *Tuina* service.
3. Facilitate teaching and training for undergraduate students.
4. More projects on experience inheritance from senior Chinese medicine doctors.
5. Establish interdisciplinary research team and conduct clinical research on integrative medicine.
6. Promote concept of Chinese medicine in disease prevention for the community.

4 Future development of integrative medicine in Hong Kong

In Hong Kong, a Chinese medicine department similar to that of HKU-SZ Hospital is being formed in Gleneagles Hong Kong Hospital, and is expected to start operating with the hospital in 2017. In that time, the model of integrated traditional Chinese and Western medicine in HKU-SZ Hospital will be implemented in Hong Kong and provide integrative medical service for the public.

5 Competing interests

The authors declare no competing interests.

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• Medical Education

The evolution of integrative medical education: the influence of the University of Arizona Center for Integrative Medicine

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ABSTRACT

The University of Arizona Center for Integrative Medicine (AzCIM) was founded in 1994 with a primary focus of educating physicians in integrative medicine (IM). Twenty years later, IM has become an internationally recognized movement in medicine. With 40% of United States' medical schools having membership in the Academic Consortium for Integrative Medicine and Health it is foreseeable that all medical students and residents will soon receive training in the principles and practices of IM. The AzCIM has the broadest range and depth of IM educational programs and has had a major influence on integrative medical education in the United States. This review describes the fellowship, residency and medical student programs at AzCIM as well as other significant national drivers of IM education; it also points out the challenges faced in developing IM initiatives. The field of IM has matured with new national board certification in IM requiring fellowship training. Allied health professional IM educational courses, as well as integrative health coaching, assure that all members of the health care team can receive training. This review describes the evolution of IM education and will be helpful to academic centers, health care institutions, and countries seeking to introduce IM initiatives.

Keywords: integrative medicine; integrative health; fellowships; integrative health coaching; medical education; competencies

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1 Introduction

In 1994, the University of Arizona College of Medicine embarked on an experiment in medical education. The Dean of the College of Medicine, Dr. James Dalen, authorized Dr. Andrew Weil to establish a Program in Integrative Medicine (IM) within the College of

Medicine. The primary focus of the program was to educate physicians in this new field. Twenty years later, IM has become an internationally recognized movement in medicine. It is instructive to examine how the program at the University of Arizona evolved and how it has influenced medical education.

This article will describe major milestones in the growth

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of the educational activities of the University of Arizona Center for Integrative Medicine (AzCIM) as well as other major developments in the field and the challenges faced; the information should be helpful to other academic centers and health care institutions seeking to replicate its accomplishments.

2 Evolution of integrative medical education

2.1 Early history at the University of Arizona

The seeds for the Program in IM were sown in 1975 when Dr. Weil was first asked to teach medical students. Initially invited to lecture on marijuana, he then gave lectures on drugs and addiction, alternative medicine, mind/body interactions, and placebos and healing. While his lectures were well received by the medical students, some senior faculty members were skeptical. By the 1980s, these lectures were a part of the regular curriculum. In 1993, Dr. Weil proposed the creation of a new residency in IM; foreseeing the critiques, Dr. Dalen recommended beginning with a fellowship instead.

A national advisory board was assembled and planning began to create a two-year residential fellowship in IM. While the structure was conventional in that it accepted board-certified physicians from primary care fields for a two-year onsite program, the content was intended to address gaps in medical education. Generous and visionary philanthropists supplied all funding for this new program, which assuaged concerns from the Dean's critics who complained that state funds ought not be used to develop an unproven field. The Dean took a significant risk amongst his peers as well, many of whom complained about the lack of evidence for some of the theories and practices included in the curriculum.

2.2 The residential fellowship

From 1997 to 2007 the University of Arizona Program in IM offered a two-year residential fellowship to primary care physicians. Thirty fellows participated with board certification in family medicine (9), internal medicine (8), pediatrics (5), Med-Peds (2), emergency medicine (2), preventive medicine (2), Ob-Gyn (1), and radiology (1). Between four and ten fellows were in training at any one time and received a modest stipend.

The curriculum addressed the evidence for nutrition, dietary supplements, exercise, and mind-body influences on health. Fellows deepened their understanding of the spiritual needs of patients and studied the fundamentals of traditional Chinese medicine, Ayurveda, homeopathy, manual medicine, and energy medicine. Patients were seen at the University of Arizona Medical Center in a consultative clinic and presented to a multidisciplinary case conference with both conventional and alternative medicine practitioners. Fellows learned from the

varying perspectives, researched the literature, and then developed comprehensive treatment plans for their patients. Skill-building in communication along with a focus on relationship-centered care prepared the fellows to interact in partnership with their patients. In addition, fellows spent two days together each month in facilitated reflections. This time was used to meditate, experience healing ceremonies, and investigate the ways in which medical training shapes physicians. In their clinical encounters, fellows practiced the art of medicine, attended to the role of language and motivation, and sought clues to enhance healing^[1].

The residential fellowship served to develop and refine the first academic curriculum in IM as well as to train early IM leaders who went on to establish new IM programs at other academic centers. While the residential fellowship was a transformational experience for the majority of physicians who participated, it depended on philanthropy to sustain it and could train only limited numbers of physicians. Scalability and financial sustainability were critical to the long-term needs of the field.

2.3 Adaptation to distributed learning fellowship

In 2000, a second significant educational experiment began at the University of Arizona Program in IM. The fellowship curriculum (Table 1) was adapted as an online program with three weeklong residential intensives. Initially named the Associate Fellowship, it is now called the University of Arizona Fellowship in IM.

Currently 130 fellows are trained each year. They are mostly board-certified, mid-career physicians from a full range of medical specialties. Given a commitment to interprofessional education, the fellowship also trains nurse practitioners, nurse midwives, physician assistants, and PharmDs.

Tuition now fully funds the educational program, meeting the challenge of sustainability. Unexpectedly, from the earliest classes, the fellows have described transformational experiences. A majority reports a renewed sense of calling to the ideals that originally brought them to medicine; they often make profound lifestyle changes and alter their professional practices. With over 1 060 graduates, the University of Arizona Fellowship is the largest fellowship program in the nation. Over its 15-year history it has trained academic leaders who practice at Duke, Johns Hopkins, Mayo, Scripps, UCSF, UCLA, UCSD, Yale, the National Institute of Health (NIH), the CDC, and other prestigious institutions. Academic institutions frequently fund faculty members' tuition so as to have a fellowship-trained IM faculty member on staff.

While the fellowship scaled from training four residential fellows per year to 130 and was financially sustainable, it did not address the need to embed training into conventional medical education. This would require a

**Table 1** The curriculum

Unit 1	Introduction to Integrative Medicine • IM Intake and Treatment Planning • Motivational Interviewing • Leadership • Medical Informatics	Year One
Unit 2	Nutrition: Macronutrients, Micronutrients, Diet & Meal Patterns, Phytonutrients • Anti-inflammatory Diet • Common Dietary Supplements • Botanical Medicine Foundations	
Unit 3	Spirituality and Health • Mind-Body Medicine • Integrative Approach to Mental Health • Sleep • Contemplative Care	
Unit 4	Manual Medicine • Integrative Approach to Pain Management • Integrative Approach to Rheumatology	
Unit 5	Life-enhancing Environments • Integrative Medicine Business and Legal Issues	
Unit 6	Whole Systems Introduction • Homeopathy • Naturopathy • Traditional Chinese Medicine • Ayurveda • Aromatherapy • Energy Medicine	
Unit 7	Integrative Approach to Cardiology • Nutrition & Cardiovascular Health • Integrative Approach to Diabetes	
Unit 8	Integrative Approach to Gastroenterology • Integrative Approach to Respiratory Health • Integrative Approach to Dermatology	Year Two
Unit 9	Integrative Approach to Women's and Men's Health • Environmental Medicine • Integrative Approach to Endocrinology	
Unit 10	Integrative Approach to Integrative Oncology • Nutrition and Cancer • Prostate Cancer or Breast Cancer	
Unit 11	Integrative Approach to Neurology • Reflections	

substantial IM curriculum integrated into the foundational training of physicians. While altering medical school education had been an initial goal, with a moderate degree of success at the University of Arizona, growing realization of the difficulty of adding hours to the already packed undergraduate curriculum led to a decision to focus on residency training instead.

2.4 Addressing conventional medical education: integrative family medicine

In 2004, with the support of the US Department of Education, a third major initiative brought IM training into residency education. The AzCIM developed a combined residency-fellowship program in partnership with six family medicine residency programs. To make sure the experience was generalizable, six family medicine residency programs were selected from urban and rural settings, community-based and academic programs. All six extended family medicine training from three to four years and offered one or two positions in the IM fellowship each year as an elective track. Residents applied for a spot and completed family medicine residency and the full University of Arizona fellowship within those four years. The Accreditation Council for Graduate Medical Education (ACGME) approved the extension of family medicine residency training to four years. To date approximately 50 residents/fellows have graduated; half chose faculty positions upon graduation and another quarter are working in underserved settings^[2].

We studied the model and found that despite being an

elective track, the joint program brought many advantages to the participating residencies. They were seen as innovative and enjoyed improved match rates. While only one or two residents completed the program each year, gradually the culture of the residency programs became more open to IM^[3]. As of 2015, three of the original six residencies continue to self fund the program, and a seventh joined the model.

While Integrative Family Medicine can be considered a successful initiative, as a national strategy to shift medical education it is limited. The 4th year salary became more difficult for residencies to obtain as budgets shrank. The curriculum and pacing of the fellowship, was designed for experienced, board-certified practicing physicians, as opposed to time-crunched residents who are just beginning to learn clinical medicine. Also, the cost of the fellowship was a challenge to several sites. Finally, the model was not scalable, given the limited fellowship spots available each year.

2.5 Scaling residency training

In 2008, a fourth major milestone was initiated to address the national need for an IM residency (IMR) training model^[4]. With funds from forward thinking philanthropists and the US Department of Education, a 200-hour curriculum was developed and evaluated. No longer an elective, the eight residency programs that piloted the initiative agreed that the curriculum would be a required element of education for all entering residents at their programs. Roughly 80% of the curriculum is web-based,

in a modular format, with the remainder consisting of individual on-site activities. The curriculum was designed to meet the needs of physicians in training and was divided into courses that can be adapted to the schedules of individual institutions. The results of a needs assessment of residents, faculty, and residency program directors as well as IM competency development informed the content of the IMR^[5,6]. A robust evaluation was designed, and four control residency sites participated in measuring medical knowledge through standardized testing and a knowledge self-assessment. Changes in wellbeing and wellness behaviors over time were also assessed using validated scales for burnout, perceived stress, emotional intelligence, depression, mindfulness, gratitude, mood and affect^[7].

2.6 IMR results

IMR pilot site residents showed significant gains in medical knowledge from baseline to graduation on a standardized test when compared to control site residents^[8]. In addition, the self-assessment of knowledge and skills demonstrated a marked increase from start of residency to the time of graduation in the pilot residents and when compared with the control site residents. Participating IMR residents evaluated the IMR curriculum positively in meeting its learning objectives and having content with sufficient depth that was clinically relevant. In an exit survey at the completion of the residency, pilot residents stated their intention to utilize IM approaches in future practice and continue IM education after residency. Another important effect of incorporating the IMR curriculum into residency training is the increase in recruitment of quality medical students into primary care^[9]. Results from wellbeing and wellness measures are still being analyzed.

2.7 Expansion from family medicine to pediatrics

As IM matured, it became clear that pediatrics was ready to incorporate its tenets. The American Academy of Pediatrics formed a Section on Complementary, Holistic and Integrative Medicine in 2005, whose mission was to further education about complementary and integrative approaches for children^[10]. A 2015 national report estimated that 12% of US children use complementary and alternative medicine (CAM); prevalence increased to more than 50% in children living with chronic illness^[11]. Despite the high prevalence of IM use by children and their families and pediatrician interest, few training programs existed in pediatric IM. Indeed, only 16 of 143 pediatric academic programs reported offering any training in IM^[12].

The Pediatric Integrative Medicine in Residency (PIMR) program was initiated in 2012 to address this gap. PIMR is a 100-hour online educational curriculum embedded into existing residency training programs and facilitated by onsite fellowship-trained IM faculty. The national pilot program is being implemented and evaluated at five pilot sites: University of Arizona, Stanford University,

University of Chicago, Eastern Virginia Medical School/Children's Hospital of the King's Daughters, and the University of Kansas^[13]. Early adopter programs that have licensed PIMR include Vanderbilt University, Cardinal Glennon Children's Hospital, University of New Mexico, Children's Hospital of Philadelphia, and the University of Southern California. In 2015, 456 pediatric residents were enrolled in the training program.

Modeled after the IMR, the PIMR curriculum is also modular, allowing flexible use in a variety of training programs. Programs use the curriculum as a teaching tool in resident continuity clinic, during specialty electives, and as a year-long seminar series on IM.

A significant innovation in the PIMR curriculum is a unit on resident self-care. This addresses the new ACGME core competencies focused on burnout prevention and promotion of resident wellness and resilience^[14]. PIMR content also covers nutrition, dietary supplements, mind-body medicine, mental health, whole medical systems, environmental health, motivational interviewing and pediatric IM intake and treatment planning.

2.8 From IM to integrative health

While physician education has been a focus of the AzCIM, demand for intensive training for allied health professionals continues to grow. In response, the Center developed a six-month online program with a four-day residential retreat. Launched in 2014, the Integrative Health and Lifestyle program (I-HeLp) has enrolled more than 120 licensed health professionals including nurses (57), behavioral health specialists (22), social workers (20), registered dietitians (15), physical therapists (4), and a variety of other providers.

A second phase of the training, Integrative Health Coaching was launched in 2015. Evaluation of both programs is in progress and the coaching program will certify providers' coaching skills as well as prepare participants for the new national health coaching certification.

2.9 Integrative health and the underserved

In 2014, the National Center for Integrative Primary Healthcare (NCIPH) was formed by the AzCIM and the Academic Consortium for Integrative Medicine and Health in cooperation with the Health Resources and Services Administration. NCIPH is a collaborative effort across disciplines and professions whose goal is to advance the incorporation of competency- and evidence-based integrative health curricula and best practices into primary care education and practice. NCIPH has developed a set of interprofessional competencies^[15]. These integrative health competencies provide educational programs a matrix upon which they can build curriculum and experiences to offer an integrative approach to primary care. NCIPH is currently creating educational materials that will advance



the incorporation of an integrative health approach into the care of diverse patient populations in primary care settings. The educational materials will include a short introductory online course as well as patient education materials.

2.10 Circling back to medical education: the Distinction Track

In 2011, in response to increasing demand on the part of the University of Arizona medical students, as well as the desire to introduce the tenets of IM at an early stage of training, a Distinction Track in IM was proposed. Distinction Tracks are elective programs of additional study available to medical students at many schools. A combination of coursework, experiences, and/or capstone projects is required in order to graduate “with distinction”. The University of Arizona College of Medicine had tracks in research, global health, and community service at the time that the IM Distinction Track was proposed. Requirements included completion of in-depth online modules, participation in interdisciplinary patient conferences, and a capstone project or research paper. The University of Arizona College of Medicine unanimously approved the proposed IM Distinction Track in 2012. It has steadily gained popularity and in 2015, 15% of first-year medical students enrolled.

3 Notable national advances in IM

Most efforts in integrative medical education have been local. A few important exceptions that helped shape the national IM educational landscape are described below.

3.1 The Consortium

In 1999, the University of Arizona, together with eight other medical schools, founded the Consortium of Academic Health Centers for Integrative Medicine. Recently renamed the Academic Consortium for Integrative Medicine and Health, it has grown steadily with 62 current North American medical school members^[6]. Its mission is to support and mentor academic leaders, faculty, and students to advance integrative health care education, research, and clinical care; to disseminate information on rigorous scientific research, educational curricula in integrative health and sustainable models of clinical care; and to inform health care policy. The Consortium has played a critical role in advancing IM medical school curricula. Members have published papers on medical school, residency and fellowship competencies^[6,17,18], established standards for research in IM and sponsored a bi-annual conference; and helped advance the integration of complementary treatments into clinical care^[19].

3.2 Board certification

The decision to develop board certification in IM was complex. On the one hand, it was considered important

that all physicians learn the foundations of IM; on the other, growing popularity of IM in the US made it unclear whether physicians claiming to practice IM were adequately trained. Much discussion among IM faculty, practitioners, and fellows led to the realization that in the maturing field, a measure of competence was required—not just to benefit IM, but also to help the public identify physicians with demonstrated expertise. Inquiries to the American Board of Medical Specialties to consider a new board were turned down, as was a request to the family medicine residency review committee to create a certificate of added qualification.

In 2010, the AzCIM entered into negotiations with the American Board of Physician Specialties (ABPS). Established in 1952, ABPS is one of the three most prominent nationally recognized multi-specialty certifying entities in North America.

The American Board of Integrative Medicine (ABOIM) was formally founded in 2013. Founding board members are national thought leaders in IM representing diverse specialties. The content and areas of competency were determined, a validated exam was created, and in 2014 the first diplomats were awarded board certification. Beginning in 2016, eligibility for board certification will require completion of a fellowship in IM. Board members are currently defining the criteria for fellowship training programs.

3.3 National Center for Complementary and Alternative Medicine educational initiatives

From 2000 to 2003 the National Center for Complementary and Alternative Medicine (NCCAM) at the NIH funded 15 projects to incorporate CAM information into the curricula of conventional health professions. The goal was to accelerate the integration of CAM and conventional medicine^[20]. The challenges at the time were considerable: to develop successful strategies given already dense curricula; to create authoritative resources about the risks and benefits of CAM; and to identify appropriate roles for CAM practitioners in educating conventional health professionals. Two NIH NCCAM projects that continued beyond the initial funding are a faculty development model and a student leadership development course.

3.4 Faculty development

Developing faculty expertise in IM is critically important for many academic programs. A novel training was developed at the University of Michigan with the NIH R25 grant, maintained with philanthropy and fees, and replicated by another academic institution. The University of Michigan Faculty Scholars Program (FSP) in Integrative Healthcare is an interdisciplinary professional development program for faculty and teaching staff. The FSP prepares faculty to incorporate theoretical, scientific,

and clinical information related to complementary, alternative, and integrative therapies into their respective disciplines. The program meets one day per month in person, and requires completion of a curriculum, research or clinical service project related to integrative health. Scholars receive mentoring by the University of Michigan IM program faculty.

3.5 Developing student leadership

A national medical student initiative was initiated in 2003 through a collaborative effort between the American Medical Student Association (AMSA) Foundation and the NIH grant-sponsored Educational Development in Complementary and Alternative Medicine Leadership Training Program. The Leadership Training began as a weeklong summer program designed to foster the development of aspiring medical student leaders in complementary, alternative and integrative medicine. Twenty medical students from across the country gathered for workshops and leadership skill enhancement with a focus on personal self-healing, wellness, and community development. Students then committed to implement a project to promote IM at their respective medical schools.

To date, more than 200 medical students have attended. In 2010, a collaboration between AMSA, the Kripalu Center, the University of Connecticut School of Medicine, and the Academic Consortium for Integrative Medicine and Health relaunched the program as Leadership and Education Program for Students in Integrative Medicine or LEAPS.

4 Discussion

The AzCIM's growth, range, depth and breadth of educational programs are unique to the time and context of the development of the field of IM in the US. Still, other institutions and countries can learn from its experience as they seek to implement and grow their own initiatives. While skepticism was common in the Program's early years, it is notable that there was unanimous support from the College of Medicine at the time that the University of Arizona Board of Regents conferred Center of Excellence status on it in 2008. This was due to the rigor of the AzCIM's educational programs, the innovative inter-institutional collaborations, and its internationally recognized success.

Online education was in its infancy in medical education when the AzCIM initiated its fellowship in 2000. Creating a mostly online fellowship was a fruitful gamble. The online platform made it possible to partner with eager learners and gifted faculty anywhere in the world. The technology also made it possible for the field to link all content to citations validating the evidence base for IM. Unlike local initiatives in which high resistance in a department or institution could stall an initiative,

residency and fellowship training in IM could now easily penetrate those fields and institutions that were ready to participate.

Medical student education was an initial high priority for the AzCIM, and many efforts were made to bring lectures in IM into a variety of courses. Ultimately, we found that targeting residency was a better strategy. Residents serve as role models for medical students, their curriculum is not as densely packed, and their work-hour ceilings make online education a valuable strategy to capture all learners. Broad incorporation of IMR was facilitated by linking it to new residency requirements — including professionalism, cultural competency, and ethical issues. The online platform made it easy to show regulators how these topics are covered in residency training.

Over time, the enthusiasm and passion of medical students at the University of Arizona led to the more in-depth distinction track experience. At the University of Arizona, students formed their own IM club, asked AzCIM faculty members to serve as advisors, designed unique learning opportunities, and eventually created a groundswell that led to an expansion of the medical student elective, and to the development of the IM Distinction Track.

Medical students have frequently been the initiators of IM programs across the nation. They often take an active role in their education, and effectively make their needs known to the deans and administrators of the Colleges of Medicine. Similarly, residents have been able to drive incorporation of IMR at their institutions.

A synergism between the fellowship and IMR programs became apparent almost at once. Fellowship-trained faculty knew of the high quality of the online educational curriculum and championed the residency training program at their institutions. Institutions without trained IM faculty often simultaneously licensed the IMR and enrolled a faculty member in the University of Arizona fellowship. Today, all 65 IMR and PIMR programs have a University of Arizona fellowship-trained faculty member in a leadership role.

The development of a certifying board in IM is an important step toward assuring uniformity of curriculum and high educational standards for the field. While most leaders in the field welcomed board certification in IM, it challenged the field's commitment to interdisciplinary collegiality. Nurses, nurse practitioners, physician assistants, and others bemoaned the fact that the certification exam was only open to physicians. While the ABOIM favors certification of all IM practitioners, each field has its own unique skills, qualifications, and governing boards, and thus needs its own certifying board.



5 The future of IM education

With 40% of US medical schools having membership in the Academic Consortium for Integrative Medicine and Health it is realistic to expect that soon all medical students will receive foundational training in the principles of IM. The steadily growing number of primary care residency programs that incorporate the 200-hour IM curriculum bodes well for post-graduate education that fully addresses health promotion, prevention, and lifestyle approaches that reduce the risk and incidence of chronic disease. Allied health programs and integrative health coaching address the training needs of the entire health care team. Fellowship programs train physicians, nurse practitioners, and physician assistants who wish to achieve advanced integrative skills to approach a broad range of conditions. And, the new ABOIM assures that standards will remain high.

While significant work remains at all levels of medical education, as well as for faculty development, it is possible to foresee a time when IM is broadly practiced and valued as the most comprehensive and cost-effective way to care for patients.

6 Competing interests

The authors declare no competing interests.

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• Medical History

The use of Chinese herbal drugs in Islamic medicine

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ABSTRACT

This paper investigates some of the ways that Chinese medicine has been transferred to the Western world and to Islamic territories. During the Golden Age of Islam (8th to 13th century CE), the herbal drug trade promoted significant commercial and scientific exchange between China and the Muslim world. Chinese herbal drugs have been described by medieval Muslim medical scholars such as Tabari (870 CE), Rhazes (925 CE), Haly Abbas (982 CE), Avicenna (1037 CE) and Jurjani (1137 CE). The term *al-sin* (the Arabic word for China) is used 46 times in *Avicenna's Canon of Medicine* in reference to herbal drugs imported from China. Cinnamon (*dar sini*; "Chinese herb"), wild ginger (*asaron*), rhubarb (*rivand-e sini*), nutmeg (*basbasa*), incense tree wood (*ood*), cubeb (*kababe*) and sandalwood (*sandal*) were the most frequently mentioned Chinese herbs in Islamic medical books. There are also multiple similarities between the clinical uses of these herbs in both medical systems. It appears that Chinese herbal drugs were a major component of the exchange of goods and knowledge between China and the Islamic and later to the Western world amid this era.

Keywords: history of medicine, Chinese medicine; Islamic medicine; herbal drugs; Golden Age of Islam

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1 Introduction

Medical histories rarely mention the relationship between the medical systems practiced in China and the Islamic world during the Golden Age of Islam (8th–13th century CE)^[1]. Indeed, the connections between the two cultures and their mutual effects upon one another have rarely been investigated. The present study reviews this exchange of

medicinal knowledge accompanying the herbal medicine trade between China and the Islamic world during the Golden Age of Islam. Medicinal literature by prominent Islamic authors, including Imam Sadiq, Rhazes, Hakim Maysari, Joveini, Hasan Ibn-e Noah Bukhari, Haly Abbas and Avicenna, was reviewed to explore written documentation of the use and trade in Chinese herbal drugs. The following questions are proposed: (1) What was the

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nature of the herbal medicine trade between China and the Middle East, during the Golden Age of Islam? (2) How did the emergence of Islam affect this connection? (3) Which Islamic medical scholars cited China as a source of herbal medicine? (4) Which Chinese herbal medicines were used by physicians in the Golden Age of Islam? (5) Besides the herbal medicine trade, what other relationships existed between Islamic and Chinese medicine?

2 Ancient trade in herbal drugs

Many medicinal herbs have been known to have various uses throughout human history. In addition to their therapeutic applications, herbs were used as spices, preservatives, perfumes and incense^[2]. The literature on this subject most often refers to the trading of such herbs as the “spice trade”^[3]. India’s Southwest coast was a major center for the spice trade starting around 3000 BCE^[4].

The incense route was another conduit for the trade of medicinal herbs during this era^[5]. This route flourished in the ancient world (7th century BCE to 2nd century CE) as a link between the Mediterranean Sea and Eastern and Southern sources of medicinal herbs and spices. This route stretched from Mediterranean ports to Egypt, through Northeast Africa and Arabia, to India and beyond. Land routes for incense from Southern Arabia to the Mediterranean flourished from 700 through 100 BCE.

Another prominent route was the Silk Road, or Silk Route, which was a series of trade routes and paths developed for trade in silk and other goods among Asian countries as well as the Western world; these trade routes also promoted cultural interaction and exchange among merchants and their countries^[6]. It linked traders, merchants, pilgrims, monks, soldiers, nomads and urban dwellers from China to the Mediterranean Sea throughout history. The Silk Route extended more than 6 000 km by land and by sea from its inception during the Han Dynasty^[7]. Beside the exchange of goods and culture, the Silk Route was a way for the transmission of disease pandemics such as the Black Death^[8,9]. The trade in medicinal herbs was also popular along this route.

3 Effect of the emergence of Islam on herbal drug trade routes

The religion of Islam arose in 610 CE^[10]. In less than one century, its territory had expanded from Northeast Africa to the borders of China^[11]. In 751 CE, Tang troops and troops from the Abbasid Caliphate troops fought in the Talas River valley and the Tang forces were defeated^[12]. This defeat, however, did not sever commercial and cultural relations between China and the Caliphate. On the contrary, Chinese artisans, brought to Samarkand after the battle

of Talas as prisoners of war, introduced paper-making to Muslims^[13]. After this era and for much of the Middle Ages, the Islamic Caliphate monopolized much of the trade conducted along the Silk Road^[14].

4 Islamic scholars citing China as a source of herbal drugs

A number of Muslim scholars have referred to China as a source of herbal drugs. It is important to note that the term “China” in their books differs from modern China. The word is used to refer to all lands and islands to the east of India, including modern Malaysia and Sri Lanka^[15]. China is called *Chin*, *Machin*, *al-Sin*, *Khata* and *Khotan* in medieval Islamic literature. It was a symbol of a far and out-of-reach land, as the prophet Mohammad says: “Seek for knowledge, even if it is in China”^[16]. China also has a symbolic role in Islamic literature as a source of silk, portraiture, perfumes, such as musk, and beautiful statues. It was also known as the source of medicinal herbs. Some important scholars who have referred to Chinese herbal medicines are introduced below.

Imam Jafar Ibn-e Mohammad-e Sadiq (702–765 CE; Medina, Saudi Arabia) was the first Muslim scholar to write about the trade in herbal drugs between nations, in his treatise *al-Ahliladj* (*Myrobalan*, in Arabic)^[17]. While discussing how the roots of medicine originate in revelations from God, he says: “How did humans know to make a compound drug from different plants, such as myrobalan from India, mastic from Rome, musk from Tibet, cinnamon from China.”^[17]

Mohammad-e Zakaria-ye Razi (854–925 CE; Rey, Iran), commonly known as Rhazes, was a Persian philosopher, chemist and physician. He was the chief physician in Rey and Baghdad hospitals. He was known as the father of Islamic medicine^[18]. Rhazes wrote a treatise on colic and mentioned cinnamon as a Chinese drug. He says: “There are different types of cinnamon, but the best type is Chinese cinnamon. Another type with lower quality is known as Gharanfol.”^[19]

Hakim Maysari (?–936 CE; Khorasan, Iran)^[20] was another Muslim medical scholar who discussed the use of Chinese drugs. His work *Daneshnameh-ye Pezeshki* (*Medical Encyclopedia*) is the oldest collection of medical poetry in *Farsi* (Persian language)^[21]. In one part he writes: “I have heard that it is better to use Chinese mamiran (celandine) in ophthalmic drugs”^[22].

al-Akhawayni Bukhari (Joveini) (?–983 CE; Bukhara, medieval Persia)^[23] was a Persian scholar who is best known for his only surviving medical treatise, entitled *Hidayat al-Mutallemin Fi al-Tibb* (*A Scholar’s Guide to Medicine*). This was the first medical text written in *Farsi Dari* (New Persian)^[24]. In this book he noted the use of

Chinese cinnamon as a treatment for epileptics: “The Chinese cinnamon and caraway should be added to his diet, lamb should be used as his meat. He should not use garlic, leek and celery in his diet. They may induce his convulsion.”^[25]

Hasan Ibn Nohe Bukhari (?–991 CE) composed the first medical dictionary for the Muslim world, *al-Tanvir (Enlightening)*. In it, he devoted a chapter to the topic of “How to process Persian rivand (rhubarb) to produce the same effect as Chinese rivand”^[26].

Ali Ibn al-Abbas al-Majusi (Haly Abbas, 930–994 CE)^[27] mentioned a Chinese source drug called zabad (a mineral from sea water) in his epic work *Kamil as-Sinat-Tibbiyya (Complete Book of the Art of Medicine)*^[28]. He writes: “There are three types of zabad; ... the second type is from China and is the best. It is similar to linseed. The third type is from India and is medium sized. Zabad has hot and dry temperament. It dispels the thick phlegm from the joints and knee.”^[29]

Ibn Sina (Avicenna, 980–1037 CE) is the most prominent of Persian Muslim physicians^[30]. He has written in depth about Chinese drugs in his medical treatises. He has described about 20 drugs “imported from China” or as being of the “Chinese type” in volume 2 of his medical encyclopedia, translated as *The Canon of Medicine*. Avicenna was born near Samarkand^[31], which is located near the southern Silk Route that passed through Bukhara. It is not surprising that he made use of medicine that was imported from China.

The most important drugs mentioned in the Canon as being imported from China are cinnamon, wild ginger, musk, croton, myrobalan, nutmeg, camphor and cubeb^[32] (Table 1).

5 Chinese herbs in the present day of the Islamic world

Many of the herbs mentioned here were imported from China throughout the medieval era, and are still on the list of imports from China into Islamic countries. The Islamic countries such as Iran, United Arab Emirates and Turkey also continue to play an important role as a route for the movement of Chinese herbs to the European countries, as in the medieval period^[33]. Herbs that are used as spice are among the most popular imported herbal materials from China to these countries; these include cinnamon, ginger, capsicum, nutmeg and aniseed^[33,34].

6 Discussion

The basic concepts taught in medical schools of China and the Islamic world have many similarities. For example, both systems believe that there is a natural power that heals disease and that the physician should aid this power, not oppose it. The concept that the human body is a small model of the universe is also a shared tenet within both

Table 1 Herbs cited in Islamic medical treatises as imported from China

English common name	Scientific name	Islamic medicine name	Chinese name	Temperament Chinese/ Islamic (4 grade)	Similar clinical indications in Islamic and Chinese medicine
Wild ginger	<i>Asarum</i>	اسارون	细辛属 (Xixin)	Warm/hot 3	Relieve visceral and joint pain, diuresis
Nutmeg	<i>Myristica fragrans</i>	بسیاسه	肉豆蔻 (Roudoukou)	Warm/hot 2	Astringent, chronic diarrhea and dysentery
Cinnamomum	<i>Cinnamomum cassia</i>	دارصینی	肉桂 (Rougui)	Hot/hot 3	Dispel internal cold from the stomach and uterus
Croton	<i>Croton tiglium</i>	دند	巴豆 (Badou)	Hot/hot 4	Edema, ascites
Myrobalan	<i>Terminalia chebula</i>	املیلج	诃子 (Hezi)	Bitter/cold 1	Astringent chronic diarrhea and dysentery
Camphora	<i>Cinnamomum camphora</i>	کافور	樟脑 (Zhangnao)	Hot/cold 3	Alleviate pain; treat sores and boils, toothache
Cubeb	<i>Piper cubeba</i>	کیابه	荜澄茄 (Bichengqie)	Warm/hot 2	Colic and diarrhea
Incense tree	<i>Aquilaria sinensis</i>	عود	土沉香 (Chenxiang)	Warm/hot 2	Wheezing, and vomiting
Santalum	<i>Santalum album</i>	صندل	檀香 (Tanxiang)	Warm/cold 2	Abdominal and chest pain
Rhubarb	<i>Rheum officinale</i>	راوند	大黄 (Dahuang)	Cold/hot 2	Hemoptysis, jaundice
Alpinia	<i>Alpinia officinarum</i>	خولنجان	高良姜 (Gaoliangjiang)	Hot/hot 2	Colic pain



Islamic and Chinese philosophies of medicine. The four elements that form the basis of Islamic medicine are similar to five elements that form the basis of Chinese medicine. Both systems classified individuals by temperament into phenotypic groups^[35].

The use of herbal drugs for specific diseases in both cultures also has multiple similarities. For example, Chinese physicians use musk as an aromatic stimulant to revive those who fall unconscious with a high fever caused by acute infectious diseases. Muslim physicians also stress its value as an aromatic stimulant and describe its tonic effect on the brain and heart. Ginger is considered to be an analgesic in both systems. Cinnamon is used to dispel internal cold in patients with gastrointestinal problems and to promote or induce menstruation for women with amenorrhea and dysmenorrhea in both medical schools^[36]. These similarities in the basics of the two systems and the use of similar drugs in response to similar indications suggest the historic connections between these two ancient medical schools.

One influence of Chinese medicinal practices on Muslim medicine may be dated to the translation of a Chinese medical book to Farsi. *The Tanksuq Nameh (Book of Valuable Information)* is also known as *Tibb-e ahl-i khata (Medicine of the Chinese)* and was translated by a team under the supervision of Khajeh Rashiduddin Fazlollah (1241–1318 CE, Hamedan), a physician and a minister to Mahmud Ghazan, the seventh ruler of the Mongol Ilkhanate^[37]. It appears to be the first, and unfortunately the last, direct translation of a Chinese medical book to a Muslim language. In the introduction to this translation, Khajeh Rashiduddin recorded the motive behind the translation. He says: “It is true that we possess much precise knowledge in our medicine, but Chinese have much knowledge that we do not possess.”

Some similarities between basic and practical concepts of Chinese and Islamic medicine predate this translation by many centuries. The herbal drug trade between China and Western Asian countries, which spread to Islamic territory after the expansion of Islam, appears to be the root of the connections between these two ancient medical schools. Considering the transfer of the Muslim scientific and medical knowledge to the Europe in the medieval era, it seems likely that these exchanges also transferred some influences of Chinese medicine to the west.

7 Conflict of interests

There is no conflict of interest.

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• Review

Phytochemistry and pharmacology of ornamental gingers, *Hedychium coronarium* and *Alpinia purpurata*: a review

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ABSTRACT

In this review, the phytochemistry and pharmacology of two ornamental gingers, *Hedychium coronarium* (butterfly ginger) and *Alpinia purpurata* (red ginger), are updated, and their botany and uses are described. Flowers of *H. coronarium* are large, showy, white, yellow or white with a yellow centre and highly fragrant. Inflorescences of *A. purpurata* are erect spikes with attractive red or pink bracts. Phytochemical investigations on the rhizomes of *H. coronarium* generated research interest globally. This resulted in the isolation of 53 labdane-type diterpenes, with little work done on the leaves and flowers. Pharmacological properties of *H. coronarium* included antioxidant, antibacterial, antifungal, cytotoxic, chemopreventive, anti-allergic, larvicidal, anthelmintic, analgesic, anti-inflammatory, anti-urolithiatic, anti-angiogenic, neuropharmacological, fibrinogenolytic, coagulant and hepatoprotective activities. On the contrary, little is known on the phytochemistry of *A. purpurata* with pharmacological properties of antioxidant, antibacterial, larvicidal, cytotoxic and vasodilator activities reported in the leaves and rhizomes. There is much disparity in terms of research effort within and between these two ornamental gingers.

Keywords: herbal drugs; plant extracts; molecular mechanisms of pharmacological action; pharmacology; Zingiberaceae; ginger; review

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1 Introduction

Gingers are herbaceous plants of the family Zingiberaceae with aromatic rhizomes that are widely cultivated for use as herb, spice or condiment^[1]. Inflorescences are terminal with fragrant flowers borne on leaf shoots or on erect shoots emerging from rhizomes. The genus *Hedychium* J. Koenig of the sub-family Hedychieae comprises 40–50 species that occur throughout Asia and the Pacific^[2]. The genus *Alpinia* Roxb. of the sub-family Alpinieae is the largest, most

widespread and the most taxonomically complex, with 230 species occurring throughout tropical and subtropical Asia^[3]. In China, there are 28 species of *Hedychium*, of which 18 are endemic, and 51 species of *Alpinia*, of which 35 are endemic^[4]. Species of *Hedychium* and *Alpinia* dominate the under-storey of forests, with many cultivated as spices, condiments, ornamental and medicinal plants; some species have become naturalized.

In this review, the chemical constituents and pharmacological properties of plant extracts and essential oils from *Hedychium coronarium* and *Alpinia purpurata*

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are updated, focusing on the description of their botanical origins and potential uses. Of the two ornamental ginger, *H. coronarium* has recently been reviewed^[5], including the phytochemical and pharmacological properties of *Hedychium*^[6], and the therapeutic properties of *Alpinia*^[7,8].

2 *Hedychium coronarium*

2.1 Botany and uses

Hedychium coronarium J. Koenig (butterfly ginger or 姜花 Jianghua), belonging to the sub-family Hedychieae, grows up to 2 m tall, forming dense clumps^[2,4]. The species is native to Myanmar, northeast India and southern China. Rhizomes are stout, fleshy and strongly aromatic. Leaves are large, oblong to lanceolate and borne on either side of thick green stems. Flowers are large, showy, white, yellow or white with a yellow centre and highly fragrant (Figure 1). The corolla tube is slender with long and curved stamens.

The plant parts of *H. coronarium* have various traditional medicinal uses. In Malaysia, leaves are boiled and consumed to treat indigestion^[2]. Leaves are taken with betel nut to ease abdominal pain. In Thailand, boiled leaves are applied to relieve stiff and sore joints. Rhizomes are consumed as stimulant and carminative. A decoction of the stem is gargled for tonsillitis. Flowers are eaten as a vegetable, worn as garland in Hawaii and Japan, and used as a source of perfume. In Vietnam, rhizomes are used for the treatment of inflammation, skin diseases, headache and rheumatic pain^[9]. In Brazil, a hot water infusion of the leaves is consumed to treat hypertension or as a diuretic^[10]. Rhizomes of *H. coronarium* are used for the treatment of headache, diabetes, inflammation and rheumatic pain in traditional Chinese medicine^[11]. In Ayurvedic medicine,



Figure 1 Flowering plant of *Hedychium coronarium*
Photo by Forest & Kim Starr.

the species is used as febrifuge, tonic, excitant and anti-rheumatic^[12]. In Madhya Pradesh, India, the intensive harvesting of *H. coronarium* plants from the forests of Dindori and Anuppur by the local people has led to the species being endangered^[13]. Practitioners of Ayurvedic medicine in the state use the essential oil from rhizomes in perfumery and the flowers to prepare an eye tonic.

2.2 Phytochemistry and pharmacology

Much research has focused on the phytochemistry of *H. coronarium* rhizomes, resulting in the isolation of many labdane-type diterpenes. Characterized by a bicyclic skeleton, they possess a broad spectrum of bioactivities. The chemistry and biological activities of labdane-type diterpenes have been reviewed by Demetzos and Dimas^[14].

In the last 27 years (1988–2015), at least 16 publications^[9,15–29], authored by scientists from nine countries and three continents reported the isolation of a total of 75 compounds from rhizomes of *H. coronarium*. Of these compounds, 53 (71%) were labdane-type diterpenes and the remaining 22 (29%) were sesquiterpenes, diarylheptanoids, phenolics, fatty acids and steroids (Table 1). Solvents used in extraction of these compounds were methanol, ethanol, ethyl acetate, dichloromethane, chloroform and hexane. The molecular structures of some labdane-type diterpenes from *H. coronarium* rhizomes are shown in Figure 2. Despite the intensive research on the rhizome, only one paper^[30] reported the identification of compounds from flowers and there has been no documentation of compounds extracted from the leaves.

2.2.1 Plant extracts

Early investigation of phytochemical properties of the chloroform extract of *H. coronarium* rhizomes in 1988 led to the isolation of two new labdane-type diterpenes (coronarins E and F), and four known compounds (coronarins A–D)^[19]. Later, in 1991 and 1993, isocoronarin D (a new diterpene) and a trinorlabdane diterpene were identified from rhizome tissue^[27,28]. Soon after, in 1994, three new labdane-type diterpenes (labda-8(17),11,13-trien-15(16)-olide, ester of labda-8(17),11,13-trien-15-al-16-oic acid and 7- β -hydroxycoronarin B), and four known diterpenes ((*E*)-labda-8(17),12-diene-15,16-dial, coronarins B and D, and isocoronarin D) were isolated from the methanol extracts of rhizome^[22]. The diterpenes showed inhibitory effects on the increase in vascular permeability, nitric oxide production and nitric oxide synthase induction.

In 2002, three new labdane-type diterpenes, hedychilactones A–C, along with six known diterpenes were isolated from the methanol rhizome extract of *H. coronarium*^[24,26]. The diterpenes were found to inhibit acetic acid-induced vascular permeability in mice and

**Table 1 (to be continued)** Chemical constituents of rhizomes of *Hedychium coronarium*

Compound	Extraction solvent	Reference
Diterpenes		
Benzoyl eugenol	EtOH	[15,16]
(<i>E</i>)-15,16-Bisnorlabda-8(17),11-dien-13-one	CH ₂ Cl ₂	[17]
Calcaratarin A	EtOH	[18]*
Coronarin A	MeOH, CHCl ₃	[18–21]*
Coronarin B	MeOH, CH ₂ Cl ₂ , CHCl ₃	[17,19,22]*
Coronarin C	CHCl ₃	[19,23]*
Coronarin D	MeOH, AcOEt, CH ₂ Cl ₂ , CHCl ₃ , C ₆ H ₁₄	[9,17–19,22–25]*
Coronarin D acetate	CH ₂ Cl ₂	[17]
Coronarin D ethyl ether	MeOH, EtOH	[18,21]
Coronarin D methyl ether	MeOH, EtOH, AcOEt, CHCl ₃ , C ₆ H ₁₄	[9,18,21,23–25]
Coronarin E	MeOH, EtOH, AcOEt, CHCl ₃	[19,20,24,25]*
Coronarin F	CH ₂ Cl ₂ , CHCl ₃	[17,19]
Coronarins G–I	MeOH	[9]
Cryptomeridiol	C ₆ H ₁₄	[23]
Docosyl-(<i>E</i>)-ferulate	CH ₂ Cl ₂	[17]
7,17-Dihydroxy-6-oxo-7,11,13-labdatrien-16,15-olide	C ₆ H ₁₄	[23]
Eicosyl-(<i>E</i>)-ferulate	CH ₂ Cl ₂	[17]
Ethoxycoronarin D	EtOH	[15,16]
Hedychenone	MeOH, AcOEt, C ₆ H ₁₄	[23–25]*
Hedychenone dimer	C ₆ H ₁₄	[23]
Hedychicoronarins A, B	MeOH	[18]*
Hedychilactones A–C	MeOH, AcOEt	[24–26]
Hedycoronals A, B	EtOH	[21]
Hedycoronens A, B	MeOH	[20]
Hedyforrestin C	MeOH	[9]
7β-Hydroxycalcaratarin A	MeOH	[18]
β-Hydroxycoronarin B	MeOH	[22]
15-Hydroxy-11,15-epoxylabda-8(17),12-dien-16-al	EtOH	[21]
7-Hydroxyhedychenone	MeOH, AcOEt	[24,25]
16-Hydroxy-labda-8(17),11,13-trien-15,16-olide	MeOH, EtOH	[20,21]
7β-Hydroxy-(<i>E</i>)-labda-8(17),12-diene-15,16-dial	CH ₂ Cl ₂	[17]
(<i>E</i>)-7β-Hydroxy-6-oxo-labda-8(17),12-diene-15,16-dial	MeOH	[18]
Isocoronarin D	MeOH, EtOH, CH ₂ Cl ₂	[15–17, 21,22,27,28]
Isocoronarin D epimer	CH ₂ Cl ₂	[17]
Labda-8(17),13(14)-dien-15,16-olide	AcOEt	[25]
Labda-8(17),11,13-trien-16,15-olide	MeOH, EtOH	[20–22]
Labda-8(17),11,13-trien-15-al-16-oic acid ester	MeOH	[22]
(<i>E</i>)-Labda-8(17),12-diene-15,16-dial	MeOH, EtOH, CH ₂ Cl ₂	[17,18,21,22]
Methoxycoronarin D	EtOH	[16]
15-Methoxylabda-8(17),11E,13-trien-16,15-olide	MeOH	[20]
16-Methoxylabda-8(17),11E,13-trien-15,16-olide	MeOH	[20]
(+)-Nerolidol	MeOH, AcOEt	[24,25]
(<i>E</i>)-Nerolidol	MeOH	[9]
6-Oxo-7,11,13-labdatrien-17-al-16,15-olide	C ₆ H ₁₄	[23]
Pacovatinin A	C ₆ H ₁₄	[23]
Peroxyoronarin D	MeOH	[18]

Table 1 (continuation) Chemical constituents of rhizomes of *Hedychium coronarium*

Compound	Extraction solvent	Reference
Sesquiterpenes		
Hedychiol A	MeOH, AcOEt	[24,25]
Hedychiol B 8,9-diacetate	MeOH, AcOEt	[24,25]
Diarylheptanoids		
(4 <i>E</i> ,6 <i>E</i>)-1,7-Bis(4-hydroxy-3-methoxyphenyl) hepta-4,6-dien-3-one	EtOH	[21]
Hedycorofurans A–D	EtOH	[29]
Hedycoropyrans A–C	EtOH	[29]
Phenolics		
4-hydroxy-3-methoxycinnamaldehyde flavonoid	C ₆ H ₁₄	[23]
4-hydroxy-3-methoxy ethyl cinnamate	C ₆ H ₁₄	[23]
Fatty acids		
Palmitic acid	MeOH	[18]
Stearic acid	MeOH	[18]
Steroids		
Daucosterol	MeOH	[9]
Ergosta-4,6,8(14),22-tetraen-3-one	MeOH	[18]
6β-Hydroxystigmast-4-en-3-one	MeOH	[18]
6β-Hydroxystigmasta-4,22-dien-3-one	MeOH	[18]
β-Sitostenone	MeOH	[18]
β-Sitosterol	MeOH	[9]
β-Stigmasta-4,22-dien-3-one	MeOH	[18]
Stigmasterol	MeOH	[9]

Molecular structures of compounds with asterisk (*) are illustrated in Figure 2. MeOH: methanol; EtOH: ethanol; AcOEt: ethyl acetate; CH₂Cl₂: dichloromethane; CHCl₃: chloroform; C₆H₁₄: hexane.

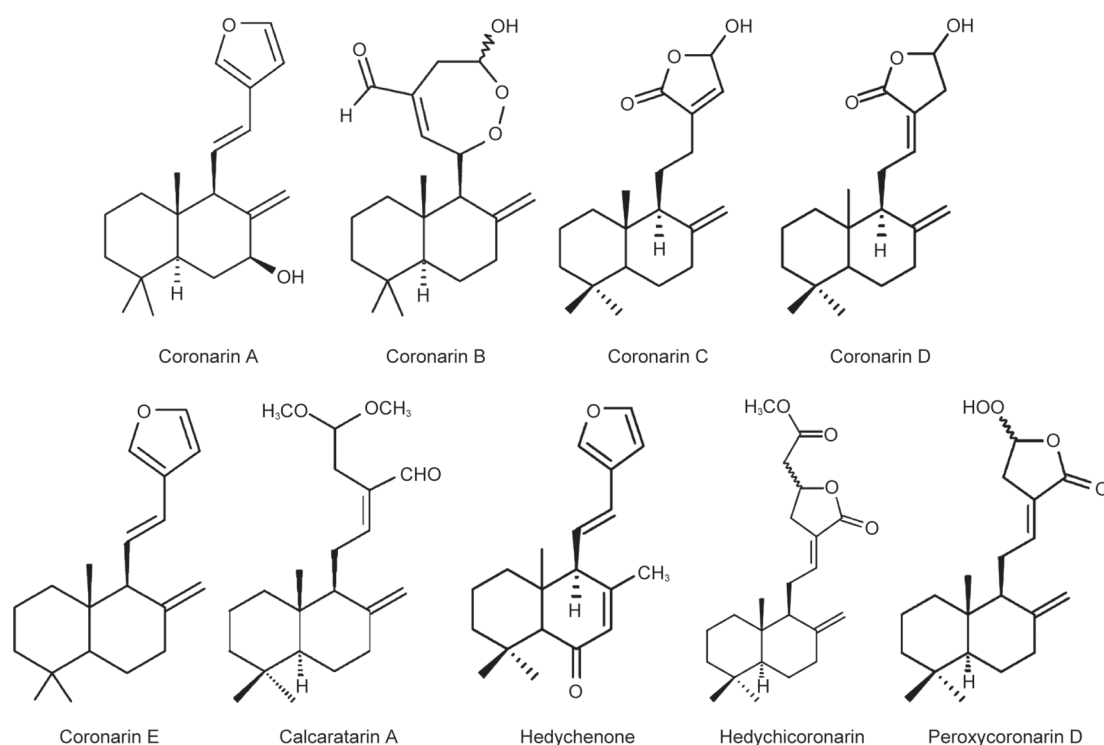


Figure 2 Some labdane-type diterpenes of *Hedychium coronarium* rhizomes

lipopolysaccharide-activated nitric oxide production in mouse peritoneal macrophages. Concurrently, two new farnesane-type sesquiterpenes (hedychiol A and hedychiol B 8,9-diacetate) were also reported, by the same group of scientists, isolated from fresh rhizomes of *H. coronarium*^[25]. Of the diterpenes isolated, hedychilactone A and coronarin D exhibited anti-allergic properties by inhibiting the release of β -hexosaminidase from RBL-2H3 cells.

Subsequently, both novel and previously described labdane-type diterpenes were isolated from rhizomes of *H. coronarium* (Table 1). They included isocoronarin D and ethoxy coronarin D^[15], 7 β -hydroxy-(*E*)-labda-8(17),12-diene-15,16-dial^[17], 6-oxo-7,11,13-labdatrien-17-al-16,15-olide and 7,17-dihydroxy-6-oxo-7,11,13-labdatrien-16,15-olide^[23], coronarins G–I^[9], hedycoronens A and B^[20], hedycoronals A and B^[21], coronarins A and D, hedychicoronarin, peroxy coronarin D, 7 β -hydroxycalcaratarin A and (*E*)-7 β -hydroxy-6-oxo-labda-8(17),12-diene-15,16-dial^[18]. From the ethanol rhizome extract of *H. coronarium*, seven new diarylheptanoids have recently been reported^[29]. Belonging to a rare class of diarylheptanoids, they were named hedycoropyrans A–C with a tetrahydropyran moiety, and hedycorofurans A–D with a tetrahydrofuran moiety.

Among the compounds isolated from *H. coronarium* rhizomes, coronarin B, coronarin D, coronarin D acetate and isocoronarin D displayed cytotoxic activity with half inhibitory concentration (IC₅₀) values less than 4 μ g/mL against selected human cancer cells^[17]. Of the compounds isolated, hedychenone coronarin C, coronarin D, 4-hydroxy-3-methoxy cinnamaldehyde and 4-hydroxy-3-methoxyethyl cinnamate exhibited potent cytotoxic activity against A-549 cells with the 50% lethal concentration (LC₅₀) values ranging from 1.3–8.0 μ mol/L^[23]. Coronarin D has been reported to inhibit the nuclear factor (NF)- κ B pathway, which led to the onset of apoptosis^[31].

Recently, isocoronarin D, methoxycoronarin D, ethoxycoronarin D and benzoyl eugenol, isolated from the ethanol extract of *H. coronarium* rhizome, have been reported to possess cancer chemopreventive activity^[16]. Isocoronarin D activated antioxidant response element with 50% effective concentration (EC₅₀) of 51 μ mol/L, while methoxycoronarin D and ethoxycoronarin D significantly inhibited NF- κ B (EC₅₀ of 7.3 and 3.2 μ mol/L), and selectively inhibited COX-1 (EC₅₀ of 0.9 and 3.8 μ mol/L), respectively. All four compounds inhibited LNCaP and HepG2 human cancer cells with IC₅₀ values ranging from 42–75 μ mol/L and from 55–79 μ mol/L, respectively.

Labdane-type diterpenes of 7 β -hydroxycalcaratarin A, calcaratarin A, coronarin A and (*E*)-labda-8(17),12-

diene-15,16-dial isolated from the methanol rhizome extract of *H. coronarium* exhibited anti-inflammatory properties *via* inhibition of superoxide anion generation and elastase release by human neutrophils with IC₅₀ values \leq 4.52 μ g/mL^[18]. Coronarin G, coronarin H and hedyforrestin C^[9], and hedycoronens A and hedycoronens B^[20] inhibited lipopolysaccharide-stimulated production of proinflammatory cytokines in the bone marrow-derived dendritic cells.

Ten labdane-type diterpenoids (hedycoronals A and B, coronarin D methyl ether, coronarin D ethyl ether, labda-8(17),11,13-trien-15(16)-olide, (12*E*)-labda-8(17),12-dien15(16)-olide, 15-hydroxy-11,15-epoxylabda-8(17),12-dien16-al, 16-hydroxylabda-8(17),11,13-trien-15,16-olide and isocoronarin D, along with a diarylheptanoid of (4*E*,6*E*)-1,7-bis(4-hydroxy-3-methoxyphenyl)hepta-4,6-dien-3-one) have been isolated from the ethanol extract of *H. coronarium* rhizome^[21]. Most of these diterpenoids showed moderate or potent cytotoxic activities against four cancer cell lines, in particular, compounds 1 and 2 inhibited HepG2 human cancer cells with 0.9 and 1.1 μ mol/L IC₅₀ values, respectively. Coronarin A and compound 3 exhibited angiogenic inhibition against human vascular endothelial cells with IC₅₀ values of 3.3 and 6.4 μ mol/L.

The antibacterial and cytotoxic activities of methanol, ethyl acetate and dichloromethane extracts prepared successively from rhizomes of *H. coronarium* have been investigated^[32]. Each of the three extracts exhibited antibacterial activity against four Gram-positive and five Gram-negative bacteria, with the dichloromethane extract showing the strongest activity. Minimum inhibitory concentration (MIC) of the extracts ranged from 8 to 128 μ g/mL. Cytotoxicity based on LC₅₀ of the extracts against brine shrimp was 35 μ g/mL for methanol, 63 μ g/mL for ethyl acetate and 56 μ g/mL for dichloromethane.

Successive hexane, chloroform and methanol rhizome extracts of *H. coronarium* were evaluated for analgesic and anti-inflammatory activities in mice and rats^[33]. At doses of 400 mg/kg, the chloroform and methanol extracts elicited 27% and 41% inhibition of writhing reflex in the acetic acid-induced writhing test, 41% and 61% increase in reaction using the tail-flick time test, and significant inhibition of paw oedema by 28% and 33% based on the carrageenan-induced paw oedema test, respectively. The analgesic and neuro-pharmacological activities of the methanol rhizome extract of *H. coronarium* were also reported in a related study, using the tail-immersion and acetic acid-induced writhing tests in mice^[34]. Using the tail-immersion test, the extract, at doses of 100, 200 and 400 mg/kg, significantly increased the pain threshold in a dose-dependent manner. In the acetic acid-induced writhing test, the extract at 400 mg/kg dose showed

a maximum of 73% writhing inhibition, which was comparable to 76% inhibition by diclofenac-Na (25 mg/kg), the standard drug.

Rhizomes of *H. coronarium* have been reported to possess anti-urolithiatic activity using calcium oxalate as experimental kidney stones^[35]. Percentage dissolution of the alcohol extract (39%) was higher than the aqueous extract (35%) and similar to cystone (39%) used as the positive control.

Among the methanol leaf extracts of 10 species and five genera of gingers belonging to the sub-family Hedychieae, *H. coronarium* had the strongest antioxidant properties based on total phenolic content of 8.2 mg gallic acid equivalent (GAE)/g and free radical scavenging of 8.1 mg ascorbic acid (AA)/g^[36]. An aqueous ethanol (50%) extract of fresh leaves of *H. coronarium* from Brazil showed hypotensive and diuretic activities in rats^[10,37].

From chloroform and acetone flower extracts of *H. coronarium*, coronalactosides I and II, and coronadiene were isolated together with (*E*)-labda-8(17),12-diene-15,16-dial, coronarins B, C and D, 15-hydroxyabda-8(17),11,13-trien-16,15-olide, 16-formylabda8(17),12-dien-15,11-olide, kaempferol 3-*O*-(2'- α -L-rhamnopyranosyl)- β -D-glucuronopyranoside and ferulic acid^[30]. Of these compounds, coronarin C and 15-hydroxyabda-8(17),11,13-trien-16,15-olide had strong hepatoprotective effects on D-galactosamine-induced cytotoxicity in mouse hepatocytes. In a comparative study of the total phenolic content and free radical scavenging activities of 69 kinds of fresh flowers in southern China, *H. coronarium* ranked moderately low with floral buds having higher values than open flowers^[38]. In India, the antifungal properties of *H. coronarium* flower extract have been reported with minimal fungicidal concentrations of 100, 500 and 750 mg/mL against *Alternaria* sp., *Fusarium* sp. and *Aspergillus flavus*, respectively^[39].

2.2.2 Essential oils

The essential oils of *H. coronarium* rhizomes from different geographical regions vary slightly in composition. Major components were 1,8-cineole (56%), β -pinene (31%) and α -pinene (11%) from Fiji^[40], and 1,8-cineole (40%) and β -pinene (25%) from Tahiti^[41]. From Mauritius, α -muurolol (17%), α -terpineol (16%) and 1,8-cineole (11%)^[42], and from Vietnam, β -pinene (24%), α -humulene (17%) and β -caryophyllene (13%)^[43], were the main components. In a given area, there may be seasonal variation in oil composition within individual plants as well as among plants of the same species. Recently, differences in the composition of oils derived from micro-propagated and conventional plants of *H. coronarium* have been identified in both leaf and rhizome tissue^[44]. Major compounds were β -pinene (27% and 15%) in the leaves and eucalyptol (48% and 34%) in the rhizomes,

respectively.

Major components of the essential oil from fresh and dried *H. coronarium* rhizomes from Kerala, India were 1,8-cineole (41% and 37%), β -pinene (10% and 17%), and α -terpineol (9% and 7%)^[45]. The aromatic oil exhibited antifungal and antibacterial properties with stronger antimicrobial activity in the oil from fresh rhizomes. Dominated by linalool (29%) and limonene (20%), the rhizome oil of *H. coronarium*, growing wild in the Kumaun region of central Himalaya, also possessed antibacterial activity against five pathogenic bacteria^[46]. Major constituents of the rhizome oil of *H. coronarium* from Taiwan, China were 1,8-cineole (37%), β -pinene (23%), α -terpineol (10%) and α -pinene (10%)^[47]. β -Pinene (34%), α -pinene (15%), 1,8-cineole (13%) and r-elemene (11%) were the main components of oil derived from the leaves. Against five fungal and four bacterial species, the rhizome and leaf oils (4 mg/disc) displayed inhibition zones of 13–26 mm and 14–26 mm. Based on percent mortality, the rhizome oil showed mosquito larvicidal activity at 2 h and 24 h with LC₅₀ values of 86 mg/L and 47 mg/L, respectively. LC₅₀ values of the leaf oil were 111 mg/L and 90 mg/L. In India, the main constituents of essential oils from rhizomes, stems and leaves of cultivated *H. coronarium* were β -pinene (39%, 39% and 44%) and α -pinene (10%, 10% and 23%), respectively^[48].

Essential oils of *H. coronarium* were tested for their inhibitory effects on coagulant and fibrinogenolytic activities induced by snake venom^[49]. Citrated human plasma was used to evaluate the clotting time, whereas changes in fibrinogen molecules were visualized by electrophoresis in polyacrylamide gel. Results showed that both the leaf and rhizome oils of *H. coronarium* interacted with venom proteases and plasma constituents, and were able to inhibit clotting; the inhibitory effect was decreased when the oils and plasma were pre-incubated prior to the addition of venoms. The anthelmintic and tranquilizing properties of the rhizome oil of *H. coronarium* have also been reported^[50,51].

Out of 131 volatile compounds identified from the flower oil of *H. coronarium*, the diffusive, sweet, spicy and floral scent was attributed to linalool, methyl jasmonate, eugenols, *cis*-jasmone, β -ionone and lactones^[52]. In a related study, the aroma of the flowers was due to linalool, methyl benzoate, *cis*-jasmone, eugenol, (*E*)-isoeugenol, jasmin lactone, methyl jasmonate, methyl epi-jasmonate, indole, nitriles and oximes^[53]. Analysis of the headspace volatile compounds of *H. coronarium* flowers yielded 39 compounds comprising monoterpene hydrocarbons (35%), oxygenated monoterpenes (34%) and sesquiterpene hydrocarbons (13%)^[54]. Major components were (*E*)- β -ocimene (29%), linalool (19%) and 1,8-cineole (15%), which contributed to the floral scent.

A total of 29 components were identified with β -trans-ocimene (28%), linalool (19%) and 1,8-cineole (11%) as main constituents of the flower oil of *H. coronarium*^[11]. Anti-inflammatory activity of the flower oil was demonstrated to significantly inhibit carrageenan-induced hind paw oedema in rats at 100 mg/kg.

3 *Alpinia purpurata*

3.1 Botany and uses

Alpinia purpurata (Vieill.) K. Schum. (red ginger or 红花姜 Honghuajiang) of the sub-family Alpinieae is a medium-sized plant up to 2 m in height^[4,55]. Leaves are deep green, alternate, sessile and oblong with a pointed apex. Leafy shoots terminate in attractive inflorescences, which are erect spikes with showy red or pink bracts (Figure 3). Rhizomes and leaf stalks are aromatic. Some individuals in the species have the ability to produce plantlets from the inflorescences^[56].

In Venezuela, inflorescences of *A. purpurata* are boiled and the hot water infusion is consumed to treat cough symptoms^[7]. In India, the rhizomes are consumed to improve appetite, taste and voice^[57]. They are also used for treating headache, rheumatism, sore throat and renal infection.

3.2 Phytochemistry and pharmacology

This species is poorly studied compared to *H. coronarium*, and from plant extracts of *A. purpurata*, only two compounds have been identified from rhizomes, five from leaves and none from flowers.

3.2.1 Plant extracts

Rhizomes of *A. purpurata* contained labda-8(17),12-diene-15,16-dial and piperine^[58]. Piperine was the first alkaloid reported from the *Alpinia* species. Phytochemical

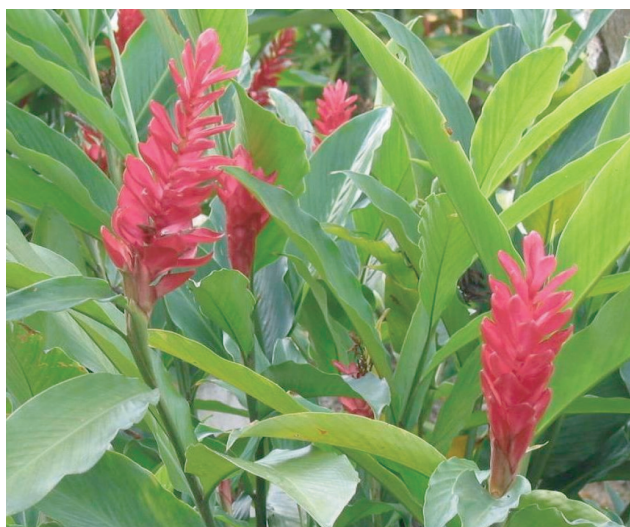


Figure 3 Flowering plant of *Alpinia purpurata*

screening of the ethanol extract of *A. purpurata* rhizome revealed the presence of flavonoids, saponins, carbohydrates, proteins, glycosides, terpenoids, resins and tannins^[59]. The screening showed substantially higher protein content in rhizomes of *A. purpurata* (70 μ g/mL) than those of *Curcuma amada* (38 μ g/mL). Antioxidant properties of *A. purpurata* rhizomes based on 2,2-diphenyl-1-picrylhydrazyl (DPPH)-free radical scavenging, ferric reducing ability and superoxide dismutase inhibition were generally higher than those of *C. amada*.

The ethanol extract of *A. purpurata* rhizome had the highest concentration of phenolics (9.5%) and flavonoids (0.85%) while the chloroform and aqueous extracts had the highest concentration of alkaloids (14.9%) and tannins (13.8%), respectively^[60]. The aqueous extract had the greatest 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid radical scavenging (IC_{50} value of 120 μ g/mL) and ferric reducing ability (IC_{50} value of 152 μ mol/L), while DPPH radical scavenging (IC_{50} value of 90 μ g/mL) was greatest in the ethanol extract.

The ethanol extract of *A. purpurata* rhizome displayed the greatest antimicrobial activity with positive inhibition against all six pathogenic bacteria and four fungi^[61]. The ranking of antimicrobial activity based on extraction solvent was ethanol > petroleum ether > chloroform; antimicrobial activity based on plant tissue was rhizomes > leaves > roots.

The total phenolic content of *A. purpurata* leaves was reported to be 16 mg GAE/g^[62]. Partitioning the crude extract with ethyl acetate and butanol followed by thin-layer chromatography and high-performance liquid chromatography resulted in the detection of kaempferol-3-O-glucuronide and rutin. The butanol extract had the highest percentage of flavonoids (94%). Fractionation of the hexane and dichloromethane leaf extracts of *A. purpurata* followed by purification using silica gel chromatography led to the isolation of 3-methoxyflavone (kumatakenin), sitosteryl-3-O-6-palmitoyl- β -D-glucoside and β -sitosteryl galactoside^[63].

Among the leaves of five *Alpinia* species screened for antioxidant properties, based on total phenolic content and free radical scavenging, *A. purpurata* ranked second with values of 12 mg GAE/g and 11 mg AA/g, respectively^[36]. Leaves of *Alpinia zerumbet* had the highest values at 20 mg GAE/g and 22 mg AA/g. Values of leaves and flowers of *A. purpurata* were comparable^[64].

Extracts and sub-extracts (64 μ g/mL) of *A. purpurata* exhibited inhibitory activity against *Mycobacterium tuberculosis*^[63]. Tested using the microplate alamar blue assay, results showed that the ethanol leaf extract possessed the highest inhibition (62%), followed by rhizome (34%) and flower (30%) extracts. Among the leaves, the dichloromethane sub-extract exhibited the

highest inhibition (72%), followed by the hexane (64%) and *n*-butanol (35%) sub-extracts.

The hydroalcoholic leaf extract of *A. purpurata* exhibited a vasodilating effect on the mesenteric vascular bed of hypertensive rats pre-treated with nor-epinephrine^[65]. The extract (60 µg/mL) induced long-lasting endothelium-dependent vasodilation with 87% efficiency. In a related study, the ethanol leaf extract of *A. purpurata* had a hypotensive effect and an endothelium-dependent vascular action on hypertensive rats^[66].

The ethyl acetate extract of *A. purpurata* leaves exhibited free radical scavenging and ferric reducing antioxidant power, and cytotoxic activity against OAW42 human ovarian cancer cells with IC₅₀ value of 130 µg/mL^[57]. The same group of scientists also reported that the extract at 2.5 mg/mL inhibited bacterial growth of *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumonia*, with inhibition zones of 5–9 mm, and displayed cytotoxic activity against PA1 human ovarian cancers cells, with IC₅₀ value of 110 µg/mL^[67].

3.2.2 Essential oils

The rhizome oil of *A. purpurata* from Fiji contained α -pinene and β -pinene, while the leaf and flower oils contained α -pinene, β -pinene and β -caryophyllene as major constituents^[40]. In samples from the Amazon, the main constituents of the leaf oil of *A. purpurata* were 1,8-cineole (22%), β -pinene (15%) and (*E*)-methyl cinnamate (13%)^[68]. The flower oil was dominated by β -pinene (28%) and α -pinene (17%). Similarly, the leaf oil of *A. purpurata* sampled from Rio de Janeiro, in Brazil, showed dominance of β -pinene (35%) and α -pinene (12%)^[69]. In Indonesia, major components of the rhizome oil of *A. purpurata* were 1,8-cineole, chavicol, β -caryophyllene and α -selinene^[70]. The oil exhibited moderate antibacterial activity against pathogenic and food spoilage bacteria, with inhibition zones of 7.2–11.2 mm in diameter and MIC values of 1.8–4.0 mg/mL.

Gas chromatography/mass spectrometry analyses of oils from flowers of red and pink cultivars of *A. purpurata* revealed the presence of 42 components, with α -pinene, β -pinene and β -caryophyllene being the major components^[71]. The oils displayed potent larvicidal activities against *Aedes aegypti* with LC₅₀ values of 81 and 72 mg/mL, respectively. Aqueous flower extracts were active against the dengue mosquito larvae with LC₅₀ values of 18% and 13%, respectively. The flower oil from the red cultivar inhibited the growth of Gram-positive bacteria of *S. aureus* with a minimum inhibitory concentration of less than 10 µg/mL.

The volatile components of essential oils from different plant parts of *A. purpurata* are listed in Table 2, based on data from five studies^[68–72]. Leaves, flowers and rhizomes yielded 62, 47 and 52 compounds, respectively. Dominant

compounds were 1,8-cineole, α -pinene and β -pinene in leaves, caryophyllene, α -pinene and β -pinene in flowers, and 1,8-cineole and chavicol in rhizomes. Compounds reported in all plant parts included borneol, caryophyllene, limonene, linalool, myrcene, nerolidiol, α -pinene, β -pinene and terpinolene.

4 Conclusion

Of the two ornamental gingers, rhizomes of *H. coronarium* have generated the most global interest with substantially less focus on leaves and flowers. Pharmacological properties of *H. coronarium* included antioxidant, antibacterial, antifungal, cytotoxic, chemopreventive, anti-allergic, larvicidal, anthelmintic, analgesic, anti-inflammatory, anti-urolithiatic, anti-angiogenic, neuro-pharmacological, fibrinogenolytic, coagulant and hepatoprotective activities. The labdane-type diterpenes of *H. coronarium* rhizomes offer promising candidates for exciting research in view of their significant medicinal properties, such as antibacterial, cytotoxic, analgesic and anti-inflammatory activities. Research programs can focus on both *in vitro* and *in vivo* analyses, and on the isolation of bioactive compounds of interest in large quantities, probably from cultivated plant resources. In contrast, there has been relatively little research on the pharmacological properties of leaves and rhizomes of *A. purpurata*, and as a result, little is known about its phytochemistry. Known pharmacological properties of *A. purpurata* include antioxidant, antibacterial, larvicidal, cytotoxic and vasodilation. It can be concluded that there is much disparity in terms of research effort within and between these two gingers.

5 Competing interests

The authors declare that they have no competing interests.

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Table 2 Volatile components of essential oils from different parts of *Alpinia purpurata*

Constituent	Leaf	Flower	Rhizome	Constituent	Leaf	Flower	Rhizome
Acetic acid	-	-	[70]	Globulol	-	-	[70]
Aromadendrene	[68]	-	-	β -Guaiene	[69]	-	-
Benzopyran	-	-	[70]	Hexyl acetate	-	[68]	-
α -Bisabolene	-	-	[70]	Hexyl hexanoate	-	[68]	-
α -Bisabolol	-	-	[70]	Hexyl 2-methyl butyrate	-	[68]	-
Borneol	[68,69]	[68,71]	[70]	γ -Himachalene	[69]	-	-
Bornyl acetate	[68,69]	[68,71,72]	-	α -Humulene	-	[72]	-
Butyl hexanoate	-	[68]	-	Humulene epoxide II	[68]	[71,72]	-
Cadina-1,4-diene	[68]	-	-	14-Hydroxy- δ -cadinene	[69]	-	-
β -Cadinene	[68]	-	-	3 α -Hydroxy-manool	[69]	-	-
7-epi- α -Cadinene	-	[68]	-	Juniper camphor	-	-	[70]
α -Cadinol	-	-	[70]	Limonene	[69]	[68,71,72]	[70]
Calamanene	[68]	-	-	Linalool	[68,69]	[68,71,72]	[70]
Camphene	[68,69]	[68,71,72]	-	Longifolene	[69]	-	-
Camphene hydrate	[68]	[68,71]	-	(E)- β -Lonone	[68]	-	-
Camphor	[68]	[68,71]	-	(E)-Methyl cinnamate	[68]	-	-
Δ^3 -Carene	[68]	-	-	(Z)-Methyl cinnamate	[68]	-	-
Carvil acetate	-	-	[70]	γ -Muurolene	[69]	-	-
Caryophyllene	[68]	[68,71*,72*]	[70]	Myrcene	[68]	[68,71,72]	[70]
Caryophyllene oxide	-	[68,71,72]	[70]	Myrtenal	[68,69]	-	-
β -Chamigrene	[69]	[71]	-	Myrtenol	[69]	-	-
Chavicol	-	-	[70*]	Nerolidol	[68]	[68,71,72]	[70]
Chavicyl acetate	-	-	[70]	Neryl acetone	[69]	-	-
1,8-Cineole	[68*]	-	[70*]	Nonanal	-	[68,72]	-
Z-Citral	-	-	[70]	Palmitic acid	-	-	[70]
α -Copaene	[68]	-	-	α -Phellandrene	[68]	[68]	-
α -Cubebene	[68]	-	[70]	β -Phellandrene	-	-	[70]
Cubenol	[68]	-	-	Piperityl acetate	[69]	-	-
Cuminaldehyde	[68]	-	-	Pinocarveol	[69]	-	-
α -Curcumene	-	-	[70]	Pinocarvyl acetate	[69]	-	-
p-Cymene	-	[68,72]	-	α -Pinene	[68,69]	[68*,71*,72*]	[70]
p-Cymen-7-ol	[69]	-	-	β -Pinene	[68*,69]	[68*,71,72*]	[70]
p-Cymen-8-ol	[68]	-	-	Pinocavone	[69]	-	-
(E)- β -Damasconone	[69]	-	-	Retinal	-	-	[70]
(Z)- β -Damascone	[69]	-	-	Sabinene	-	[72]	-
(E)-2-Decenal	[68]	-	-	Sabinyl acetate	[69]	-	-
Dillapiol	-	[68]	-	Safrole	-	[68]	-
Dimethyl-octadienyl	-	-	[70]	α -Selinene	-	[68]	[70]
Drim-8-(12)ene	-	[72]	-	β -Selinene	-	[72]	-
β -Elemene	-	[71]	[70]	(7)-epi- α -Selinene	-	[72]	-
γ -Elemene	-	-	[70]	β -Sesquiphellandrene	-	-	[70]
Elemol	-	-	[70]	α -Sinensial	-	-	[70]
Epiglobulol	-	-	[70]	Terpineol	-	[72]	[70]
Ethyl alcohol	-	-	[70]	α -Terpineol	[68,69]	[68,71]	-
Ethyl oleate	-	-	[70]	α -Terpinene	-	[72]	-
Ethyl palmitate	-	-	[70]	β -Terpinene	-	-	[70]
α -Eudesmol	[68]	-	-	γ -Terpinene	[68]	[72]	[70]
7-epi- α -Eudesmol	[69]	[72]	-	Terpinen-4-ol	[68,69]	[68,71]	-
β -Eudesmol	[68]	[71]	-	Terpinolene	[68]	[68,71,72]	[70]
γ -Eudesmol	[68]	[71]	-	Tetradecane	-	[68]	-
Eugenol	-	-	[70]	α -Thujene	-	[72]	-
(Z,Z)-Farnesal	-	-	[70]	3-Thujopsanone	[69]	-	-
Farnesene	-	-	[70]	Tricyclene	[68]	-	-
β -Farnesene	[68]	-	[70]	β -Tumerone	-	-	[70]
Farnesyl acetate	-	-	[70]	Valencene	-	[71,72]	[70]
Fenchol	[68]	[71,72]	-	Valerolactone	-	[72]	-
Fenchyl acetate	[68]	[68,71,72]	-	Viridiflorene	-	[72]	-
Geraniol	[68]	-	[70]	α -Ylangene	-	-	[70]
Germacrene D	[69]	-	-	Total	62	47	52

References with asterisk (*): dominant constituents.

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• Research Article

The effect of acupuncture on mood and working memory in patients with depression and schizophrenia

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ABSTRACT

BACKGROUND: In patients with depression, as well as in patients with schizophrenia, both mood and working memory performance are often impaired. Both issues can only be addressed and improved with medication to some extent.

OBJECTIVE: This study investigates the mood and the working memory performance in patients with depression or schizophrenia and whether acupuncture can improve these.

DESIGN, SETTING, PARTICIPANTS AND INTERVENTIONS: A pragmatic clinical trial design was used. The study was conducted in a psychiatric clinic. Fifty patients with depression and 50 with schizophrenia were randomly divided into an experimental and a waiting-list group. Additionally, 25 healthy control participants were included. Twelve weeks of individualized acupuncture treatment was used as the clinical intervention.

MAIN OUTCOME MEASURES: All patients were tested before (T1) and after (T2) acupuncture treatment on a mood scale (Beck Depression Inventory-II, BDI-II), a simple working memory task (digit span), and a complex working memory task (letter-number sequencing); the healthy controls were tested at T1 only.

RESULTS: Patients with depression scored worse than the others on the BDI-II, and patients with schizophrenia scored worse than the healthy controls. On the digit span, patients with schizophrenia did not differ from healthy controls whereas they scored worse of all on the letter-number sequencing. With respect to the acupuncture findings, first, the present study showed that the use of acupuncture to treat patients with schizophrenia was both practical and safe. Moreover, acupuncture had a positive effect on the BDI-II for the depression group, but acupuncture had no effect on the digit span and on the letter-number sequencing performance for the two clinical groups.

CONCLUSION: The clinical improvement in patients with depression after acupuncture treatment was not accompanied by any significant change in a simple working memory task or in a more complex working memory task; the same was true for the patients with schizophrenia.

TRIAL REGISTRATION: Dutch Trial Register NTR3132.

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1 Introduction

Acupuncture is increasingly used as a complementary medicine treatment for psychiatric illnesses^[1]. Previous research showed that acupuncture affects mood; a reduction of depressive symptoms in patients with depression after acupuncture treatment was revealed by a meta-analysis based on 8 randomized trials^[2]. Recently, researchers have also started to investigate acupuncture for the treatment of schizophrenia^[3] and lower positive, negative, and general symptoms in patients with schizophrenia have been reported after acupuncture treatment^[4]. However, despite its long tradition and wide applications, many questions remain; for instance, is acupuncture able to improve cognitive functioning? In studies on cognitively impaired animals, acupuncture was found to have a restoration and protection effect^[5,6]. Other studies reported improvements in cognitively impaired patients^[7,8].

Therefore, one important cognitive process that will be investigated in the present study is working memory (WM). WM is known to play an important role in higher-level cognitive functioning^[9], like reasoning^[10] and spatial visualization^[11]. WM was originally assumed to consist of a central executive that was responsible for monitoring and coordinating two slave systems: the phonological loop and the visuo-spatial sketchpad. The phonological loop temporarily buffers spoken and written material whereas the visuo-spatial sketchpad stores and processes visual or spatial information^[12]. Later, the episodic buffer was added as a third slave system and was responsible for linking information across domains to form integrated units of verbal, visual and spatial information with time sequencing^[13]. A recent two-group, randomized, single-blind study on 90 healthy students showed that acupuncture significantly increased WM performance on an automated operation span task in comparison with the control group^[14].

If acupuncture can improve WM performance in healthy participants, it might also be beneficial for the psychiatric population. For this reason, two long-term (illness duration longer than 5 years) patient groups that were known to have decreased WM performances, namely, patients with schizophrenia^[15] and patients with depression^[16], were investigated by addressing the following research questions: what are the moods of long-term patients with depression or schizophrenia, and what are their WM performances? Also, can acupuncture change mood^[2] and WM performance^[14] in patients with depression and in

patients with schizophrenia?

The first hypothesis is that patients with depression score worse on mood than the patients with schizophrenia and the healthy controls. The second hypothesis is that both patients with schizophrenia and those with depression score worse on WM than the healthy controls. Thirdly, we hypothesize that acupuncture treatment will improve mood in patients with depression and perhaps also in patients with schizophrenia. Fourthly, the WM performance in patients with schizophrenia and in patients with depression is expected to be improved after acupuncture treatment.

2 Subjects and methods

2.1 Sample

All patients with schizophrenia were randomly divided into an experimental group and a waiting-list group, as were the patients with depression, and we used the random number generator program in Microsoft Excel for the randomization. The patients in the waiting-list group received no treatment other than their normal psychiatric treatment. According to the 10th revision of the International Classification of Diseases and Related Health Problems (ICD-10)^[17], the patients with schizophrenia were diagnosed with schizophrenia F20.0 (paranoid schizophrenia) or F20.5 (schizophrenic residuum), and the patients with depression were diagnosed with depression F33.2 (recurrent depressive disorder, current episode severe without psychotic symptoms).

2.2 Experimental design

2.2.1 Design

A pragmatic, clinical trial design^[18] was used to address the effectiveness of acupuncture as a health care intervention in treating patients with schizophrenia or depression in real clinical practice^[19].

2.2.2 Acupuncture treatment as clinical intervention

The participants were treated weekly for twelve consecutive treatments. Individualized acupuncture according to traditional Chinese medicine principles was applied after careful diagnosis^[3] by a licensed Oriental medical practitioner with more than 5 years of clinical experience^[20]. Needles were left in place for one hour after insertion.

2.2.3 Testing

The tests were completed by the patients in the experimental groups before (T1) and after (T2) acupuncture treatment. For the patients in the waiting-list groups, the tests were

also conducted twice, but without treatment. The healthy control participants only completed the tests at T1. Testing was conducted in the LVR-Klinik Bedburg-Hau by apprentices that were not informed about the rest of the project.

2.3 Operationalizations

2.3.1 Mood scale

The Beck Depression Inventory-II (BDI-II)^[21] was used to measure (depressed) mood^[22,23]. The BDI-II was found to have an excellent test-retest reliability of 0.96^[24].

2.3.2 Simple working memory task

The digit span forward subtask was conducted in order to measure WM^[25]. The digit span has a good (0.80) test-retest reliability^[26].

2.3.3 Complex working memory task

Complex span tasks^[27], like the letter-number sequencing task^[27], have been developed to measure WM capacity^[27], and its norm score was used in the present study^[28]. The test-retest reliability of the letter-number sequencing task was found to be good (0.80)^[26].

2.4 Acupuncture treatment

2.4.1 Needles

Depending on the place of needling, 0.25 mm × 25 mm or 0.20 mm × 15 mm stainless-steel single-use needles (AcuPro C, Wujiang City Cloud & Dragon Medical Device Co., Ltd., China) were used.

2.4.2 Acupuncture points that were used

An overview of the acupuncture points that were most

frequently used (> 50%) during the 12 weekly individual acupuncture treatments is given in Figure 1 for the depression group and in Figure 2 for the schizophrenia group. In total, 56 acupuncture points were used in the depression group, of which the 15 most frequently used points were Hegu (LI4) (96%), Sanyinjiao (SP6) (96%), Yinlingquan (SP9) (96%), Shenmen (HT7) (95%), Taixi (KI3) (95%), Sishencong (EX-HN1) (92%), Zusanli (ST36) (91%), Taichong (LR3) (78%), Quchi (LI11) (53%), Yanglingquan (GB34) (36%), Gongsun (SP4) (34%), Xuehai (SP10) (34%) and Lieque (LU7) (32%), whereas for the schizophrenia group, a total of 61 acupuncture points were used, of which the 15 most frequently used points were EX-HN1 (89%), SP6 (60%), SP9 (60%), LI4 (56%), KI3 (55%), LR3 (54%), HT7 (53%), Baihui (DU20) (49%), ST36 (47%), KI6 (44%), LI11 (37%), LU7 (35%), Yutang (CV18) (28%), Lidui (ST45) (23%) and Wenliu (LI7) (22%). A complete list of all acupuncture points used per individual treatment and a detailed justification for point selection^[29] can be obtained from the corresponding author.

2.5 Analysis design

Before acupuncture treatment (T1), differences between the patients with depression and the patients with schizophrenia in both the experimental and the waiting-list groups and the healthy control participants on the BDI-II^[21], digit span forward^[24], and letter-number sequencing^[28] were

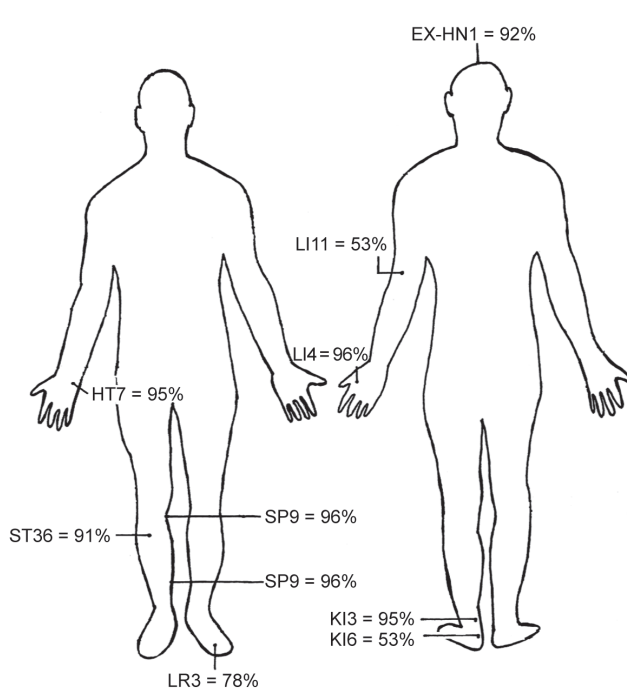


Figure 1 An overview of the acupuncture points that were most frequently used (> 50%) during the 12 weekly individual acupuncture treatments in the group with depression

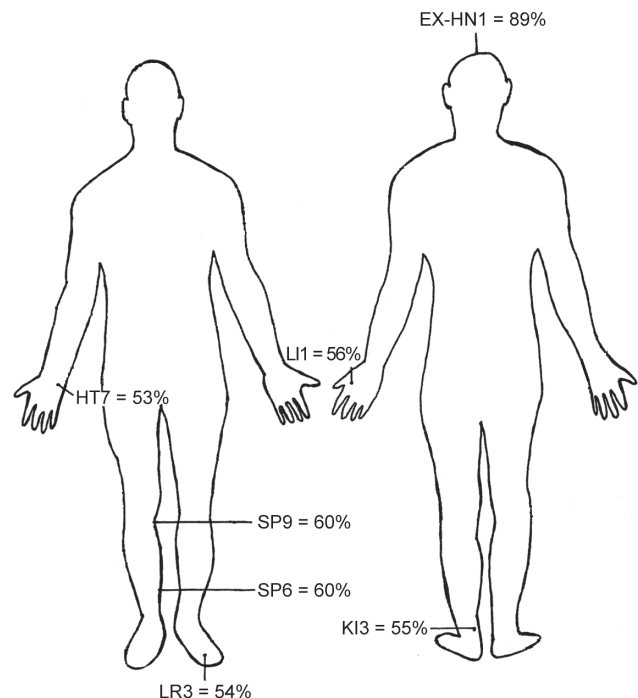


Figure 2 An overview of the acupuncture points that were most frequently used (> 50%) during the 12 weekly individual acupuncture treatments in the group with schizophrenia

analyzed by using the analysis of variance (ANOVA). For those analyses, first, patients with depression, patients with schizophrenia, and healthy controls were compared; then, further analyses were conducted for more detailed comparisons. Considering that a relatively large number of patients in the waiting-list groups had dropped out by the end of acupuncture treatment, T2, we decided against a within-group factor design. For the psychiatric groups, the results after acupuncture treatment (T2) were also pooled in order to answer the questions whether acupuncture treatment improved mood in a psychiatric population and whether group (treatment and waiting-list groups) differences existed. Next, paired *t* tests were used to establish within-group effects. Because of the difference in age that existed between the depression and the healthy control groups, an analysis of covariance (ANCOVA) was conducted in order to determine if the results were affected by this variable.

3 Results

3.1 Sample characteristics

In total, 50 patients with schizophrenia and 50 patients with depression were selected by their psychiatrists and asked to participate voluntarily; moreover, 25 healthy control participants entered the study (Table 1 and Figure 1). The patients with schizophrenia had a total score of 73.65 (standard deviation = 23.43) on the Positive and Negative Syndrome Scale (PANSS)^[30]. Exclusion criteria were addiction (other than nicotine), epilepsy or other neurological disorders, and other co morbid psychiatric disorders.

As can be seen in Table 1, no significant gender differences were observed between the two experimental groups ($P = 0.222$, partial $\eta^2 = 0.041$). However, a significant difference in age did exist (Table 1) ($P = 0.008$, partial $\eta^2 = 0.126$), and that was due to the fact that the patients with depression were significantly older than the healthy control participants ($P = 0.007$). As can also be seen in Table 1, the lengths of illness for the patients with depression and the patients with schizophrenia in the experimental groups were not different ($P = 0.171$, partial $\eta^2 = 0.039$). Thus, because both groups had lengths of illness of > 5 years, both could be classified as long-term psychiatric groups^[31]. An overview

Table 1 Participants' demographic information

Group	<i>n</i>	Gender (males/females)	Age (in years)	Length of illness (in years)
Depression experimental group	25	3/22	48.68±10.60*	7.99±5.83
Schizophrenia experimental group	25	8/17	41.80±10.30	10.00±4.30
Depression waiting-list group	25	2/23	46.32±8.12	6.75±5.21
Schizophrenia waiting-list group	25	11/14 [△]	43.96±10.87	10.70±5.47 [△]
Healthy control group	25	7/18	38.88±12.15	—

Data of age and length of illness are presented as mean ± standard deviation. * $P \leq 0.05$, vs healthy control group; [△] $P \leq 0.05$, vs depression waiting-list group.

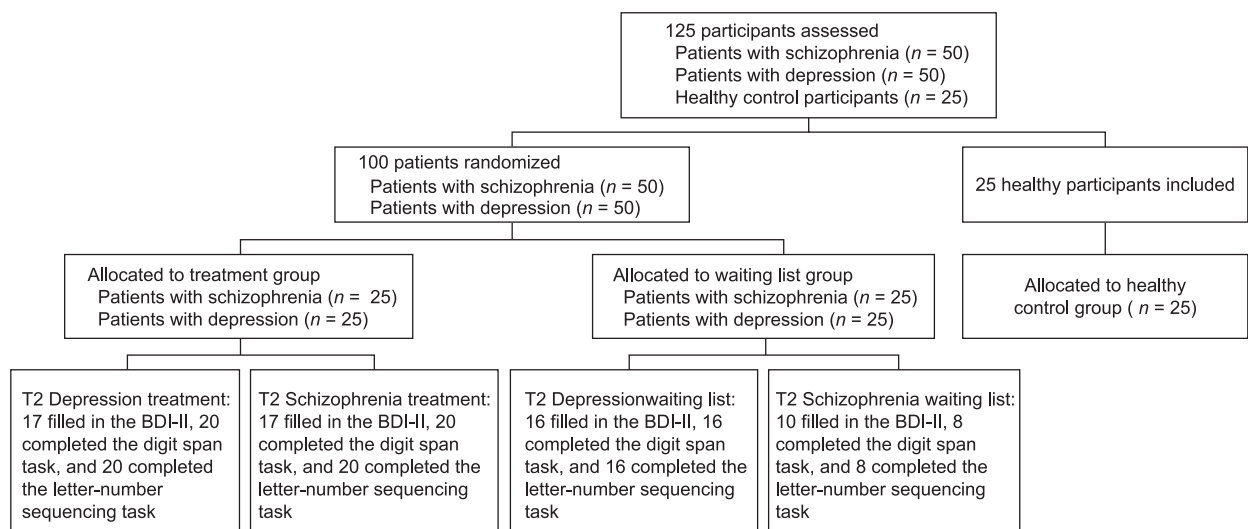


Figure 3 Flow chart

Some patients with depression or schizophrenia were unable to complete the BDI-II, the digit span task, and the letter-number sequencing task. Moreover, many patients in the waiting-list group could not be motivated to come to the after-test appointment. BDI-II: Becks Depression Inventory-II

**Table 2 (to be continued)** Overview of the medication used per individual at the start of the study specified for the experimental depression group and the experimental schizophrenia group

Group	Patients	Medication	Daily dose
Depression group	D1	Doxepin	2 × 25 mg
		Citalopram	1 × 20 mg
	D2	Citalopram	1 × 20 mg
	D3	Trevilor Retard	1 × 150 mg
	D4	Mirtazapin	1.5 × 30 mg
	D5	No medication	No medication
	D6	Zoloft	1 × 50 mg
		Amineurin	1 × 25 mg
	D7	Zoloft	3 × 50 mg
		Chlorprothixen	2 × 15 mg
	D8	Citalopram	1 × 20 mg
	D9	Lyricea	1 × 75 mg
		Doxepin	3 × 10 mg
		Diazepam	0.5 × 10 mg
	D10	Venlafaxin Retard	1 × 75 mg
		Venlafaxin	1 × 150 mg
		Seroquel Prolong	2 × 50 mg
		Chlorprothixen	2 × 15 mg
	D11	Trevilor Retard	1 × 75 mg
	D12	Doxepin	2 × 25 mg
		Citalopram	1 × 30 mg
	D13	Promethazin	1 to 2 × 25 mg
		Venlafaxin Retard	1 × 75 mg
	D14	No medication	No medication
	D15	Valdoxan	2 × 25 mg
	D16	Cymbalta	1 × 30 mg
		Doxepin	1 × 15 mg
	D17	Seroquel Prolong	1 × 50 mg and 1 × 150 mg
		Lamotrigine	1 × 50 mg and 1 × 100 mg
		Abilify	1 × 10 mg
	D18	No medication	No medication
	D19	No medication	No medication
	D20	Trevilor Retard	1 × 150 mg
		Promethazin	1 × 50 mg
	D21	Venlafaxin	1 × 150 mg
		Melneurin	1 × 25 mg
	D22	Valdoxan	2 × 25 mg
		Circadin	1 × 2 mg
	D23	Citalopram	1 × 20 mg
	D24	Pipamperon	1.5 × 40 mg
Schizophrenia group		Timonil	3 × 200 mg
		Zoloft	3 × 50 mg
	D25	Trenitor Retard	1 × 37.5 mg and 1 × 75 mg
	S1	Zeldox	2 × 20 mg
		Nipolept	2 × 50 mg
		Sulpirid	1 × 300 mg
		Amitriptylin	1 × 25 mg
	S2	Orifril	2 × 600 mg
		Solian	1 × 200 mg
		Zyprexa Velotab	1 × 5 mg
	S3	Zeldox	1 × 60 mg and 1 × 40 mg
		Seroquel Prolong	1 × 50 mg
	S4	Nipolept	3 × 25 mg
		Lithium-Ion	2 × 12.2 mmol
		Zyprexa	2 × 7.5 mg
		(Fluanxol)	(every two weeks 10% 1 mL)
	S5	Zyprexa Velotab	1 × 5 mg and 1 × 10 mg
	S6	Xeplion	1 × 25 mg
		Levomepromazin	2 × 10 mg
	S7	Seroquel Prolong	1 × 400 mg
		Seroquel	1 × 100 mg
		Chlorprothixen (Xeplion)	1 × 50 mg (1 × 100 mg per month)

Table 2 (continuation) Overview of the medication used per individual at the start of the study specified for the experimental depression group and the experimental schizophrenia group

Group	Patients	Medication	Daily dose
Schizophrenia group	S8	Leponex	2×100 mg and 1.5×25 mg
	S9	Leponex	2×100 mg
	S10	Paroxetin Beta	3×10 mg
		Amisulprid	2×400 mg
	S11	Zyprexa Velotab	2×10 mg
		Clozapine Beta	1×25 mg
		Dipiperon	$1 \times$ as-needed
		Ergenyl Chrono	1.5×300 mg
	S12	Seroquel	2×25 mg
		Citalopram	1×20 mg
		Abilify	1×10 mg
		Citalogram	0.5×20 mg
	S13	Risperdal (Xeplion)	1×2 mg (1×75 mg per month)
		Pipamperon	0.75×40 mg
		Sertralin	3.5×50 mg
		Solian	2×200 mg
	S14	Trimineurin	3×25 mg
		Solvex	0.5×4 mg
		Nipolept	2×100 mg
		Melperon	25 mg as needed
	S15	Edronax	1×4 mg
		Zopiclon	0.5×7.5 mg as needed
		Risperdal	2.5×2 mg as needed
		Promethazin	1×10 mg
	S16	Fluanxol	2×5 mg
		Akineton	0.5×2 mg
		Zyprexa	1×5 mg
		Lithium-Ion	2×12.2 mmol
	S17	Nortrilen	3×25 mg
		Abilify	1×10 mg
		Zeldox	1×60 mg and 1×40 mg
		Truxal	15 mg up to $3 \times$ daily
	S18	Sertralin	2×50 mg
		Seroquel Prolong	1×200 mg
		Risperdal	2×2 mg
		Lithium-Ion	2×12.2 mmol
	S19	Cipralex	1×10 mg
		Mirtazapin	1×15 mg
		Zeldox	2×60 mg
		Seroquel Prolong	1×200 mg
	S20	Ergenyl Retard	2×500 mg
		Risperdal	2×3 mg
		Mirtazapin	1×30 mg
		Haldol	3×5 mg
	S21	Dominal Forte	1×40 mg
		Pipiperon	2×40 mg
		Pipamperon	0.75×40 mg
		Sertralin	3.5×50 mg
	S22	Solian	2×200 mg
		Trimineurin	3×25 mg
		Zyprexa (Dapotum)	2×7.5 mg (every 21 d $\times 25$ mg)
		Solian	1×400 mg
	S23	Clozapin	2.5×200 mg
		Clozapin	5×25 mg
		Lamotrigin	2×50 mg
		Risperdal	1×2 mg
	S24	Leponex (Haldol)	1×50 mg and 1.5×25 mg ($1 \times$ per month)
	S25	Zyprexa	1×5 mg and 1×10 mg
		Truxal	1×50 mg

D: patient with depression; S: patient with schizophrenia.

of the exact medications used for each individual is given in Table 2. None of the participants had ever received acupuncture treatment. All participants gave written consent, and the ethics committee of the Ärztekammer Nordrhein approved the study beforehand (number 2008331); moreover, the clinical trial has been officially registered under number NTR3132 at the Dutch Trial Register (see also <http://www.trialregister.nl/trialreg/admin/rctview.asp?TC=3132>). Finally, the study was conducted according to the Declaration of Helsinki^[32].

3.2 Mood

The analysis (ANOVA) of the BDI-II scores at the baseline (T1) showed a significant difference in total BDI-II score between the depression group, the schizophrenia group, and the healthy control group ($P = 0.000$, partial $\eta^2 = 0.676$). As can be seen in Table 3, patients with depression had higher BDI-II scores than both patients with schizophrenia ($P = 0.000$) and healthy control participants ($P = 0.000$); moreover, patients with schizophrenia had higher BDI-II scores than healthy control participants ($P = 0.000$).

After acupuncture treatment (T2), the patients with depression still had higher scores than the patients with schizophrenia ($P = 0.000$, partial $\eta^2 = 0.456$). With respect to the “acupuncture effect” (*i.e.*, before versus after acupuncture treatment), the analysis of the total psychiatric group scores (which was used in order to maintain as much statistical power as possible) on the BDI-II showed that those scores were significantly lower after acupuncture treatment ($P = 0.027$, partial $\eta^2 = 0.058$). An analysis (ANCOVA) revealed that age did not have a significant influence on this result ($P = 0.170$, partial $\eta^2 = 0.023$), nor

did length of illness ($P = 0.057$, partial $\eta^2 = 0.046$). Moreover, the analysis of the waiting-list groups revealed that the total psychiatric group effect after acupuncture could not be explained by the fact that the participants were tested twice ($P = 0.429$, partial $\eta^2 = 0.008$). Finally, when the depression and the schizophrenia groups were analyzed separately, the patients with depression showed significantly lower BDI-II scores after acupuncture treatment ($P = 0.014$, partial $\eta^2 = 0.141$), but the patients with schizophrenia did not ($P = 0.121$, partial $\eta^2 = 0.059$).

3.3 Simple working memory task

An analysis (ANOVA) of the digit span scores at baseline (T1) showed significant differences between the depression group, the schizophrenia group, and the healthy control group ($P = 0.003$, partial $\eta^2 = 0.148$). As can be seen in Table 4, patients with depression had lower digit span scores than healthy control participants ($P = 0.002$), but their scores did not differ from the scores of patients with schizophrenia ($P = 0.181$). Moreover, no differences in digit span scores were found between the patients with schizophrenia and the healthy control participants ($P = 0.278$). After acupuncture treatment (T2), the scores of the patients with depression again did not differ from the scores of the patients with schizophrenia ($P = 0.949$, partial $\eta^2 = 0.000$).

With respect to the “acupuncture effect”, an analysis of the total psychiatric group scores before versus after acupuncture treatment showed no significant differences ($P = 0.528$, partial $\eta^2 = 0.005$). Similarly, non-significant results on the digit span tasks performed before and after acupuncture treatment were found when the depression

Table 3 Overview of the mean BDI-II scores at baseline (T1) for the healthy control group, and both at T1 and after acupuncture treatment or after a waiting list (T2) for the depression group and the schizophrenia group

Group	BDI-II score at T1	BDI-II score at T2
Depression experimental group	30.48±9.06 ($n=25$)*	23.00±7.83 ($n=17$) [▲]
Schizophrenia experimental group	14.12±10.70 ($n=25$)* [△]	9.35±7.54 ($n=17$)
Depression waiting-list group	25.04±11.43 ($n=25$)	21.56±11.34 ($n=16$)
Schizophrenia waiting-list group	17.52±13.74 ($n=25$)	14.60±9.89 ($n=10$)
Healthy control group	2.12±2.37 ($n=25$)	—

Data are presented as mean ± standard deviation. * $P \leq 0.05$, vs healthy control group; [△] $P \leq 0.05$, vs depression experimental group; [▲] $P \leq 0.05$, vs BDI-II score at T1. BDI: Beck Depression Inventory.

Table 4 Overview of the digit span task scores at baseline (T1) for the healthy control group, and both at T1 and after acupuncture treatment or after a waiting list (T2) for the depression group and the schizophrenia group

Group	BDI-II score at T1	BDI-II score at T2
Depression experimental group	30.48±9.06 ($n=25$)*	23.00±7.83 ($n=17$)
Schizophrenia experimental group	14.12±10.70 ($n=25$)*	9.35±7.54 ($n=17$)
Depression waiting-list group	25.04±11.43 ($n=25$)	21.56±11.34 ($n=16$)
Schizophrenia waiting-list group	17.52±13.74 ($n=25$)	14.60±9.89 ($n=10$)
Healthy control group	2.12±2.37 ($n=25$)	—

Data are presented as mean ± standard deviation. * $P \leq 0.05$, vs healthy control group.

group ($P = 0.194$, partial $\eta^2 = 0.039$) and the schizophrenia group ($P = 0.740$, partial $\eta^2 = 0.003$) were analyzed separately.

3.4 Complex working memory task

As shown in Table 5, analysis (ANOVA) of the scores on the letter-number sequencing task at baseline (T1) showed significant differences in those scores between the depression group, the schizophrenia group, and the healthy control group ($P = 0.000$, partial $\eta^2 = 0.192$). The patients with schizophrenia scored significantly lower than both the patients with depression ($P = 0.025$) and the healthy control participants ($P = 0.000$). This difference between two psychiatric groups had vanished by the end of acupuncture treatment (T2) ($P = 0.122$, partial $\eta^2 = 0.062$).

With respect to the “acupuncture effect” (*i.e.*, before versus after acupuncture treatment), the analysis of the total psychiatric group scores before versus after acupuncture treatment showed no improvements after acupuncture treatment ($P = 0.264$, partial $\eta^2 = 0.014$). Finally, the analysis of the subgroups after acupuncture treatment did not reveal significant differences on the letter-number sequencing task for the depression group ($P = 0.719$, partial $\eta^2 = 0.003$) and for the schizophrenia group ($P = 0.178$, partial $\eta^2 = 0.042$).

4 Discussion

This pragmatic, clinical trial^[18] investigated mood^[2] and WM^[14] in a psychiatric sample and attempted to determine if acupuncture might be able to improve mood and WM. Differences were found between the psychiatric groups and the healthy control group, but not on all measures. The most relevant acupuncture outcome was that acupuncture treatment significantly improved BDI-II^[21] scores in patients with depression.

We confirmed the low BDI-II scores for the depressed population, and interestingly, the patients with schizophrenia showed signs of better mood, although their average score was still below what is considered clinically relevant. In line with our hypothesis, after 12 weeks of acupuncture treatment, patients with depression reported an improved mood on the BDI-II, which is one

of the most widely used instruments for measuring the severity of depression^[33]. Moreover, previous research has shown that the BDI-II has excellent test-retest reliability^[24]. This finding and our waiting-list results (*i.e.*, no significant difference in BDI-II between two measurements) are further support for the hypothesis that the different scores before and after acupuncture treatment are a result of the acupuncture intervention. This finding is in line with previous acupuncture research on depression and confirms that acupuncture is effective in reducing depressive symptoms^[34]. In contrast to the depression group, and against our hypothesis, however, the patients with schizophrenia did not show lower BDI-II^[21] scores after acupuncture treatment. An explanation for their stable mood might be the fact that the patients with schizophrenia have much lower scores on the BDI than the depressed group, mean = 14.12 versus mean = 30.48, so those patients do not have much room for improvement. This is in sharp contrast to the data on the patients with depression who had plenty of room for improvement as a result of acupuncture. Nevertheless, as can also be seen in Table 3, both experimental groups show a reduction of more than 4.5 points in the total BDI-II score, and although this result may not be significant for the group with schizophrenia, it may have clinical relevance, particularly for the individual patient in daily clinical practice, and warrants further research.

As can be seen in Table 4, the WM results showed that, at baseline, only the patients with depression scored lower than the healthy controls on the simple WM task; the patients with schizophrenia surprisingly scored within the normal range. This was not expected because research has shown that patients with schizophrenia show relatively stable problems within broad neurocognitive domains^[35], and evidence even exists for ongoing neurocognitive deterioration as the disease progresses^[35]. Moreover, cognitive dysfunction is seen as a biomarker that is even visible in healthy relatives of patients with schizophrenia^[36]. On the other hand, patients that have been ill for a long time have been studied less, which might explain the unexpected performance^[37]. In contrast to our hypothesis, the scores on the digit span

Table 5 Overview of the mean scores on the letter-number sequencing task at baseline (T1) for the healthy control group, and both at T1 and after acupuncture or after a waiting list (T2) for the depression group and the schizophrenia group

Group	BDI-II score at T1	BDI-II score at T2
Depression experimental group	8.28±1.97 ($n=25$)*	9.10±2.20 ($n=17$)
Schizophrenia experimental group	9.28±1.90 ($n=25$)	9.05±2.70 ($n=17$)
Depression waiting-list group	9.00±2.65 ($n=25$)*	9.00±2.45 ($n=16$)
Schizophrenia waiting-list group	9.26±1.76 ($n=25$)	8.38±2.77 ($n=10$)
Healthy control group	10.16±1.77 ($n=25$)	—

Data are presented as mean±standard deviation. * $P \leq 0.05$, vs healthy control group.

did not change after acupuncture treatment, showing that acupuncture did not influence performance on a simple WM task. The reason for this might be that the baseline scores (particularly for the schizophrenia group) were already in the normal range, and as a result, acupuncture could not further improve them.

The results of the complex WM task revealed that, at baseline, as we expected, the patients with schizophrenia performed worse on the letter-number sequencing task^[28] than both the patients with depression and the healthy controls (see also Table 5); however, surprisingly, the patients with depression did not perform worse than the healthy controls. More importantly, after acupuncture treatment, no WM improvements were found in both clinical groups. Future research with a more complex WM task (such as the reading span task^[38]) is needed in order to exclude the possibility that the letter-number sequencing task, which was used in the present study, did not tap enough processing elements (in addition to the storage elements)^[38], and as a result, no effect of acupuncture on the complex WM task could be found.

One of the possible mechanisms of action is that the (beneficial) effect of acupuncture in depression may work via the neurotransmitters dopamine, norepinephrine, and serotonin that are often reported in the literature because of their role in depression^[39–41]. Previous research found evidence for the fact that in cases of shortage, acupuncture causes the release of dopamine, norepinephrine, and serotonin^[42–44]. As a result, a modulating and normalizing effect occurs^[45], leading to a decrease of depressive symptoms^[46]. Another possible mechanism of action is that the (beneficial) effect of acupuncture in depression might work via an indirect working mechanism, namely the improvement of sleep^[45]. Sleep disorders are often reported in patients with depression or schizophrenia^[47]; moreover, sleep was found to improve after acupuncture in patients with depression or schizophrenia^[3,4]. Future research on those possible mechanisms of action is needed in order to firstly explain the (beneficial) effect of acupuncture in depression that is reported in the literature^[46] and was found in the present study and secondly, why no beneficial effect of acupuncture was found in schizophrenia in this study. Perhaps the effects of strong medication use of the patients with schizophrenia with the acupuncture treatment may be an explaining factor. Especially in typical antipsychotics, dopamine D2 receptors are blocked^[48], thereby possibly interfering the mechanism of acupuncture.

The present study has several limitations. For instance, based on the present study, how long the improved mood in patients with depression lasts after finishing the acupuncture treatment is unclear. Future studies (with follow-up measurements) on the clinically relevant long-term effects of acupuncture treatment are, therefore, needed^[49].

Another limitation of our study is that all participants were on medication during the study (see also Table 2), and medications could not be stopped due to ethical reasons^[50] (raised by the ethics committee of the Ärztekammer Nordrhein). Finally, the sample sizes in the present study are relatively small^[51] due to practical reasons^[52]. Therefore, in future research, larger clinical groups need to be investigated in multiple-center studies.

5 Conclusions

The clinical improvement in the patients with depression after acupuncture treatment was not accompanied by significant changes in either a simple or a more complex WM task; the same was true for patients with schizophrenia.

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7 Authors' disclosure statement

The authors declare no conflicts of interest.

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Submission Guide

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● Research Article

Effects of wet-cupping on blood pressure in hypertensive patients: a randomized controlled trial

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ABSTRACT

BACKGROUND: Although cupping remains a popular treatment modality worldwide, its efficacy for most diseases, including hypertension, has not been scientifically evaluated.

OBJECTIVE: We aimed to determine the efficacy of wet-cupping for high blood pressure, and the incidence of the procedure's side effects in the intervention group.

DESIGN, SETTING, PARTICIPANTS AND INTERVENTIONS: This is a randomized controlled trial conducted in the General Practice Department at King Abdulaziz University Hospital, Jeddah, Saudi Arabia, between May 2013 and February 2014. There were two groups (40 participants each): intervention group undergoing wet-cupping (hijama) in addition to conventional hypertension treatment, and a control group undergoing only conventional hypertension treatment. Three wet-cupping sessions were performed every other day.

MAIN OUTCOME MEASURE: The mean systolic and diastolic blood pressures were measured using a validated automatic sphygmomanometer. The follow-up period was 8 weeks.

RESULTS: Wet-cupping provided an immediate reduction of systolic blood pressure. After 4 weeks of follow-up, the mean systolic blood pressure in the intervention group was 8.4 mmHg less than in the control group ($P = 0.046$). After 8 weeks, there were no significant differences in blood pressures between the intervention and control groups. In this study, wet-cupping did not result in any serious side effects.

CONCLUSION: Wet-cupping therapy is effective for reducing systolic blood pressure in hypertensive patients for up to 4 weeks, without serious side effects. Wet-cupping should be considered as a complementary hypertension treatment, and further studies are needed.

TRIAL REGISTRATION: *ClinicalTrials.gov* Identifier NCT01987583.

Keywords: blood pressure; hypertension; cupping therapy; randomized controlled trials

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1 Introduction

Hypertension is an important health problem, rated globally as the number one mortality risk factor in 2004^[1]. Worldwide, approximately 40% of adults over age 25 are reported to be hypertensive^[2]. In Saudi Arabia, the overall prevalence is 25.5% among 15–64 year olds^[3]. Despite its prevalence, a real cure for the disease has yet to be discovered. All currently available anti-hypertension medications control blood pressure (BP) for a very limited time, never exceeding a single day, rather than actually being curative. Additionally, these medications are also associated with side effects and increased costs for the patients. As a result, the World Health Organization (WHO) stated that, currently, a more suitable long-acting, single dose/day anti-hypertension medication without side effects, that can also reverse the complications of hypertension, is still needed^[4]. Thus, the search continues for a new anti-hypertension remedy.

Cupping is an ancient healing method that has been practiced for centuries in many parts of the world. Cupping therapy can be divided into two broad categories, dry- and wet-cupping. Dry-cupping is the process of using a vacuum on different areas of the body in order to collect blood in that area without any incisions^[5]. Wet-cupping (or hijama in Arabic) is the process of using a vacuum at different points on the body, along with the use of incisions (small, light scratches made using a razor), to remove what was previously termed as ‘harmful blood’ (this represents accumulated blood that is located just beneath the skin surface)^[5].

Although cupping remains a popular treatment modality in many parts of the world, its efficacy for most diseases, including hypertension, has not been scientifically studied. A recent systematic review involved searching 15 databases, without language restrictions, and included all relevant trials through June 2009^[6]. Only 2 studies met the inclusion criteria, and only one assessed the effects of wet-cupping. In that study, 35 patients with acute hypertension were included, and all patients underwent three wet-cupping sessions every other day on the GV14 (Dazhui) acupuncture point; there was no control group. After a single wet-cupping session, acute hypertension improved in 71% of the patients^[7]. The authors of the systematic review concluded that there was no strong evidence suggesting that cupping is an effective treatment for hypertension, and that further research is required^[6]. A recent randomized controlled trial (RCT) assessed the efficacy of wet-cupping for the treatment of hypertension. The protocol randomly divided 42 participants into intervention and control groups. After 6 weeks of follow-up, a comparison of the mean BP differences between the intervention and control groups showed a significant difference in systolic BP (SBP), but not in diastolic BP (DBP)^[8].

Thus, further evidence is needed to establish the efficacy

of wet-cupping for lowering high BP. The present study investigated the efficacy of wet-cupping in lowering BP in hypertensive patients, and assessed the incidence of side effects among the treated participants.

2 Materials and methods

The present RCT was conducted in the General Practice Department at King Abdulaziz University Hospital, Jeddah, Saudi Arabia, between May 2013 and February 2014. *The Declaration of Helsinki* was followed and ethical approval was given by the Unit of Biomedical Ethics at King Abdulaziz University before data collection.

This two-armed study involved an intervention group, undergoing wet-cupping (hijama) in addition to conventional hypertension treatment, and a control group undergoing only conventional hypertension treatment. The study could not be blinded because blinding was impossible for this procedure, unlike that for dry-cupping^[9].

2.1 Participants

The participants were included in the study if they had high (grade I or II)^[4] BP at the time of the study (SBP ≥ 140 mmHg and/or DBP ≥ 90 mmHg). For patients with diabetes mellitus, high BP was defined as SBP ≥ 130 mmHg and/or DBP ≥ 85 mmHg^[10]. Patients were required to be 19–65 years old, and both men and women were included. Patients were excluded if they had grade III hypertension (SBP ≥ 180 mmHg and/or DBP ≥ 110 mmHg), very high added risk according to the WHO hypertension management guidelines^[4], or secondary hypertension, or were pregnant. Patients who had undergone dry-cupping, wet-cupping, or acupuncture within the previous six months were also excluded, as were those who required anti-hypertension medication dose or type changes within the follow-up period.

2.2 Randomization and ethical considerations

After checking for eligibility, written informed consent was obtained, and the participants were randomized into the treatment or control group using block randomization method. To preserve concealment, the randomization was performed using sealed opaque envelopes, such that neither the patient nor the observer could predict the group to which a participant was assigned. The randomization process and patient enrolment into their groups were done by the prime investigator. Patient confidentiality was ensured throughout the study, and participants were free to exit the study whenever they desired.

2.3 Intervention

The hijama procedure, performed on intervention group patients, involved cleaning the target area with an alcohol swab, placing the cup over the area, and starting suction. The cup was then gently removed, and five very superficial incisions were made parallel to each other. After creating

the incisions, the cup was placed over the same area and the suctioning was repeated. The cupping procedure was repeated approximately three times without repeating the incisioning, and then the area was cleaned and dressed. Hijama was performed at four sites (Figure 1). The first site was between the two scapulae, opposite the T1–T3 scapular spine. This is the recommended site for treatment of hypertension in an RCT previously done in Iran^[7]. This area is called Al-Kahil in Arabic. The second site was located on the seventh cervical vertebra. This site was used in the uncontrolled observational study performed in China on the efficacy of wet-cupping for hypertension^[6], and it is called GV14 in Chinese medicine. The other two sites were on both sides of the neck. They are located two fingers posterior to the angle of the mandible on both sides, just below the skull bone, on the hairline. These two areas are called Al-Akhdaain in Arabic, and they were added because they are recommended areas in Islamic literature for general healing along with Al-Kahil^[11].

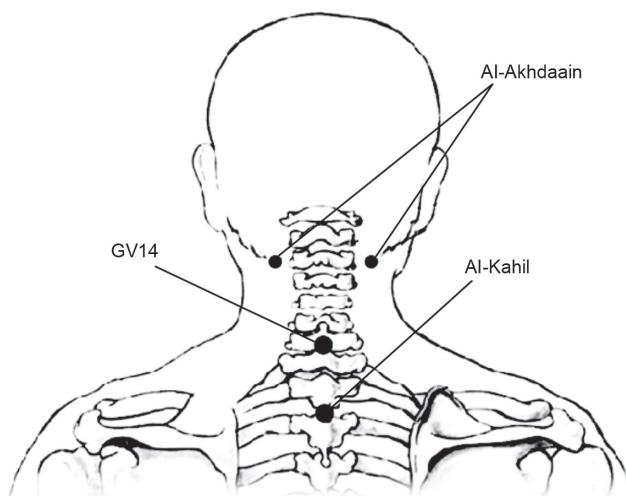


Figure 1 Wet-cupping treatment points

The hijama sessions were repeated 3 times, with a rest day between sessions^[6,8]. In Islamic literature, hijama is recommended to be done on days 17, 19, and 21 of the lunar calendar month; these sessions were performed accordingly^[11].

2.4 Outcome measures

The main outcome measure in this study was BP measurements. For BP measurement, we followed the BP measurement standards recommended by the *Saudi Hypertension Management Guidelines*^[10]. According to these guidelines, the patient rested for 3–5 min before the BP was measured; measurements were performed on both arms during the initial visit. The patient avoided consumption of nicotine or caffeine for 1 h prior to the BP measurement. An appropriate cuff size was used — either a standard or large cuff — according to the

upper arm circumference of the participant. All BP measurements were performed with the patient in a sitting position, using a validated, automatic oscillometric sphygmomanometer (705IT; Omron, Kyoto, Japan) to minimize observer bias^[12–14]. The instructions provided by the device manufacturer were carefully followed for the measurements. BP was recorded at least twice during each visit, and the mean value was documented.

The potential side effects were those previously published^[15]. The occurrence of side effects was evaluated immediately after each hijama session (immediate effects), as well as 4 weeks after the sessions (late effects). The percentage of side effects experienced due to the hijama procedure was calculated.

2.5 Sample size

Based on figures from a previous pilot study^[16], we used a standard deviation of ± 15.9 mmHg to calculate the sample size necessary to detect a difference of 10 mmHg between the groups. A sample size of 80 participants, equally divided between the intervention and the control groups, was determined to be sufficient to detect a 10-mmHg change in SBP with 80% power and $\alpha = 0.05$.

2.6 Statistical analysis

Statistical analyses were conducted using SPSS, version 16.0 (IBM, Armonk, NY, USA). BP comparisons were performed between the intervention and control groups at baseline, 4 weeks after intervention, and 8 weeks after intervention using unpaired Student's *t*-test analyses. A second BP comparison was conducted within each group using a paired *t*-test. *P* values < 0.05 were considered significant. Mean BP differences, with 95% confidence intervals, were reported. The percentage of patients experiencing any hijama-related side effect was calculated. An intention-to-treat analysis was used to consider participants lost to follow-up.

3 Results

3.1 Participants' inclusion and exclusion process

During the recruitment period, 318 participants were screened to check for the primary eligibility criteria; 180 individuals were excluded, and 58 refused to participate in the study. The remaining 80 participants were recruited into the study and randomized into the intervention (40 participants) and control (40 participants) groups. Three participants did not attend the 4-week follow-up session and 7 did not attend the 8-week follow-up session. Further, 1 participants from the intervention group and 3 from the control group were excluded at the last follow-up appointment because of changes in their anti-hypertension medications (Figure 2).

3.2 Baseline characteristics' comparison

The intervention and control groups had well-matched

baseline characteristics, without statistically significant differences between any of the baseline variables, except for fasting blood sugar levels, which were significantly higher in the intervention group than in the control group ($P = 0.022$). The baseline BP measurements were not significantly different (Table 1).

3.3 Conventional anti-hypertension treatment details

Most of the participants in this study were already taking

anti-hypertension medications, including 28 in the intervention group and 33 in the control group. The number of anti-hypertension medications used by the participants was not significantly different between the two groups. In addition, the intervention and control groups were compared regarding the class of anti-hypertension medications taken by the participants. There were no significant differences between the two groups in that area (Table 2).

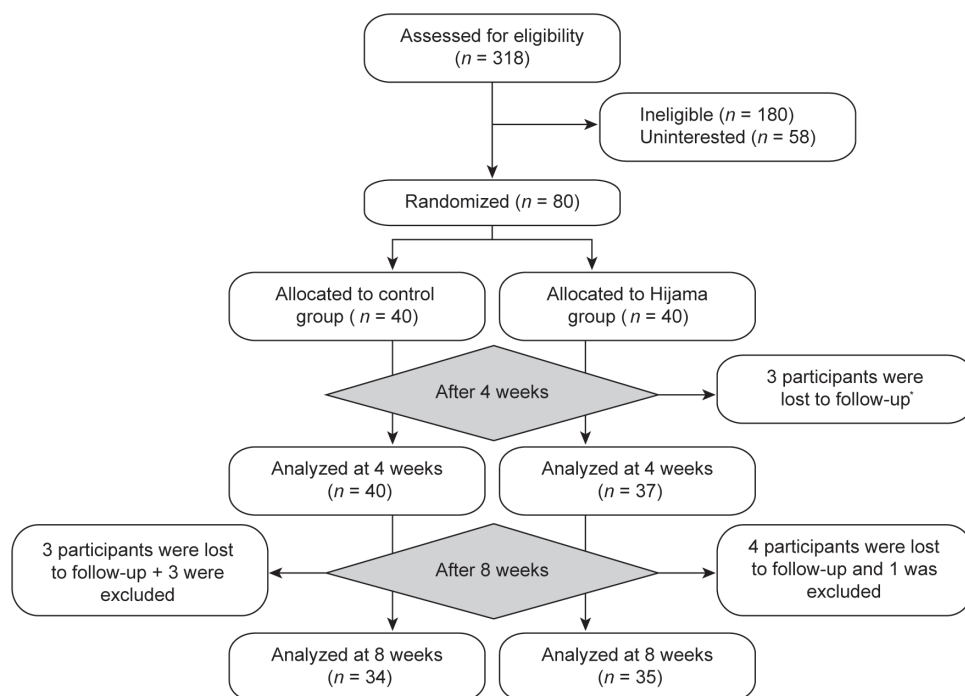


Figure 2 Participant flow chart

*The 3 participants who were lost to follow-up at 4 weeks showed up at the 8 weeks.

Table 1 Comparison of the participants' baseline characteristics

Baseline characteristic	Intervention group (n=40)	Control group (n=40)	P value
Mean age, years (\pm SD)	52.0 (\pm 9.4)	53.8 (\pm 9.5)	0.409
Male:female ratio	13:27	11:29	—
Diabetes, n (%)	25 (62.5)	23 (57.5)	0.648
Hyperlipidemia, n (%)	26 (65)	22 (58)	0.434
Mean body mass index, kg/m ² (\pm SD)	32.1 (\pm 6.2)	33.2 (\pm 6.4)	0.444
Currently smoking, n (%)	3 (7.5)	2 (5.1)	1.0
Family history of premature cardiovascular disease deaths, n (%)	2 (5)	3 (7.7)	0.675
Mean fasting blood sugar, mmol/L (\pm SD)	8.5 (\pm 4.1)	6.6 (\pm 1.8)	0.022
Mean low-density lipoprotein level, mmol/L (\pm SD)	3.2 (\pm 1.0)	2.9 (\pm 0.7)	0.160
Mean high-density lipoprotein level, mmol/L (\pm SD)	1.3 (\pm 0.4)	1.3 (\pm 0.5)	0.621
Mean creatinine level, mmol/L (\pm SD)	62.0 (\pm 18.0)	54.6 (\pm 24.0)	0.145
Mean potassium level, mmol/L (\pm SD)	3.9 (\pm 0.7)	5.5 (\pm 8.9)	0.283
Mean thyroid-stimulating hormone level, mIU/L (\pm SD)	4.8 (\pm 12.2)	2.9 (\pm 2.5)	0.345

SD: standard deviation.

During the entire follow-up period, any participant who had changed his or her anti-hypertension medication was excluded from the study, as mentioned before. The participants' compliance with their anti-hypertension medication schedule was measured, using a validated tool^[17-19], at the beginning and end of the study. In addition, histories of the use of anti-hypertensive herbal treatments or other concomitant medications were also obtained. These variables were compared between the intervention and the control groups using chi square or Fisher's exact tests; no significant differences were observed.

3.4 Blood pressure changes during follow-up

At the 4-week follow-up visit, BP measurements were repeated for both groups. The mean SBP and DBP after intervention in the hijama group were significantly different (paired *t*-test) from those at baseline ($P = 0.000$ and 0.042 , respectively). In the control group, there were also significant differences (paired *t*-test) in the SBP and DBP compared to those at baseline ($P = 0.016$ and 0.003 , respectively). When comparing the mean BP readings between the two groups after 4 weeks of follow-up (Student's *t*-test), there was a significant difference in SBP values ($P = 0.046$) but not in DBP values ($P = 0.681$). The mean difference in SBP values between the two groups after 4 weeks of follow-up was -8.4 mmHg (95% confidence interval, -16.7 to -0.1).

After 8 weeks of follow-up, significant differences persisted within the hijama group, for SBP and DBP ($P = 0.002$ and 0.004 , respectively, compared with those at

baseline). Similar results were also found for both SBP and DBP in the control group ($P = 0.036$ and 0.022 , respectively, compared with those at baseline). When comparing the mean BP readings (independent Student's *t*-test) between the two groups, after 8 weeks of follow-up, the differences in the SBP and DBP values were not significantly different between the groups ($P = 0.129$ and 0.881 , respectively) (Table 3).

3.5 Assessment of factors that may affect the participants' blood pressure

As various factors may alter BP results, we repeated the comparisons several times while accounting for these factors. One such factor was the amount of blood collected during hijama. This factor was not included in the original protocol; therefore, a cut-off value for high and low volumes of collected blood was not prospectively determined. The amount of blood collected was recorded using the symbols +, ++, +++ or +++, and was only recorded during the third hijama session. Nevertheless, we believe that this factor should be accounted for, and hence, the amount of blood collected was estimated as accurately as possible. Therefore, the hijama group was divided into two groups — the lower amount of blood extracted (LABE) group included those with + and ++ (estimated to represent less than 50 mL per session), and the higher amount of blood extracted (HABE) group included those with +++ and +++++ (estimated to represent more than 50 mL per session). Thereafter, both the HABE and LABE groups were compared with the control group. When the LABE

Table 2 Comparison of the class of anti-hypertension medications taken by the participants

Anti-hypertension medication class	Intervention group (<i>n</i> =40)	Control group (<i>n</i> =40)	<i>P</i> value
Angiotensin-converting enzyme inhibitors (%)	18 (45%)	13 (32.5%)	0.251
Calcium channel blockers (%)	10 (25%)	14 (35%)	0.329
Thiazide diuretics (%)	3 (7.5%)	8 (20%)	0.105
β ₁ Receptor antagonists (%)	3 (7.5%)	6 (15%)	0.481
Angiotensin-II receptor antagonists (%)	3 (7.5%)	5 (12.5%)	0.712
Loop diuretics (%)	1 (2.5%)	0 (0%)	1.0

Table 3 Blood pressure comparisons between the intervention and control groups after 4 and 8 weeks of follow-up, and the changes from baseline within each group

(Mean ± standard deviation, mmHg)		
Group	Systolic BP	Diastolic BP
Hijama group		
At baseline (<i>n</i> =40)	152.0 ± 10.7	85.0 ± 7.9
After 4 weeks (<i>n</i> =37)	140.0 ± 17.7*	82.0 ± 9.9
After 8 weeks (<i>n</i> =35)	143.0 ± 19.8	81.0 ± 10.4
Control group		
At baseline (<i>n</i> =40)	157.0 ± 11.3	86.0 ± 6.4
After 4 weeks (<i>n</i> =40)	149.0 ± 18.5	81.0 ± 12.2
After 8 weeks (<i>n</i> =34)	150.0 ± 15.8	82.0 ± 12.1

* $P < 0.05$, vs control group. BP: blood pressure.

group was compared with the control group, the BP results were not significantly different. However, there was a significant difference in SBP at the 4-week follow-up visit when the HABE group was compared with the control group. Finally, we compared the LABE group with the HABE group. The SBP and DBP values were significantly different between these two groups after 4 weeks of follow-up (Table 4).

The other factors that may affect blood pressure outcome were also assessed, including gender, number of hijama sessions, body mass index, compliance with the anti-hypertension medication therapy, and the class of the anti-hypertension medication taken by the patient. None of these factors had a significant effect on the blood pressure outcomes.

3.6 Assessment of wet-cupping's side effects

Serious side effects were not observed in the hijama group. Most of the mild side effects were experienced immediately after hijama and lasted for few hours, but never for more than 48 h. This excludes hijama-site pruritus, which appeared 1–2 d after the session and lasted for a few days. The most common immediate side effects were headache, followed by hijama-site pruritus, dizziness, and feeling tired and sleepy after hijama. Wound infections

were not observed 1–2 weeks after the intervention in any of the participants. After 8 weeks of follow-up, the only remaining side effect was a mildly hyperpigmented scar at the hijama site in 10 participants (27.8% of the hijama group) (Table 5). In addition, all of the mentioned side effects were compared between the HABE and LABE groups after the 3rd session, because the amount of blood was only recorded at that time, and there was no significant difference between the two groups (Table 6).

4 Discussion

The results of the present study showed a significant difference in SBP measurements (–8.4 mmHg) between the intervention and control groups after 4 weeks of follow-up. After 8 weeks of follow-up, the hijama effect had disappeared, leaving no significant BP difference between the intervention and control groups. The positive results reported in this study are consistent with those of Zarei *et al*^[8] who also reported a significant difference in SBP values between the intervention and control groups after 6 weeks of follow-up. Therefore, hijama produces an effect that lasts for 4–6 weeks.

Table 4 Comparison of the BP values between the higher amount of blood extracted (HABE) group and the lower amount of blood extracted (LABE) group

Measurement (mmHg)	HABE group	LABE group	Mean difference between the two groups	P value
Systolic BP at baseline	150 (±12.2)	156 (±9.2)	5.9 (–1.5–13.2)	0.114
Diastolic BP at baseline	85 (±8.3)	85 (±8.1)	–0.2 (–5.8–5.5)	0.957
Systolic BP after 4 weeks	133 (±12.5)	146 (±20.8)	2.8 (0.4–25.1)	0.043
Diastolic BP after 4 weeks	78 (±11.1)	85 (±7.5)	7.0 (0.2–13.7)	0.044
Systolic BP after 8 weeks	141 (±14.2)	144 (±24.4)	3.0 (–13.1–19.2)	0.704
Diastolic BP after 8 weeks	81 (±10.6)	81 (±11.7)	0.2 (–8.5–8.9)	0.963

After 4 weeks of follow-up, the group with a higher amount of blood extracted included 15 participants, whereas the group with a lower volume of blood extracted included 20 participants. After 8 weeks of follow-up, the group with a higher amount of blood extracted included 12 participants, whereas the group with a lower volume of blood extracted included 17 participants. BP: blood pressure.

Table 5 Frequency of adverse events immediately after each hijama session

Side effect	After session 1	After session 2	After session 3	Total frequency	Percentage (based on total number of sessions)
Headache	4	3	6	13	11.8%
Hijama-site pruritus	0	3	5	8	7.3%
Dizziness	5	2	0	7	6.4%
Tired and sleepy	1	3	3	7	6.4%
Nausea	2	0	1	3	2.7%
Pain at hijama site	0	0	2	2	1.8%
Same-day insomnia	1	1	0	2	1.8%
Vomiting	1	0	0	1	0.9%
Bullae formation	0	0	0	0	0%

Table 6 Comparison of the wet-cupping side effects between the higher amount of blood extracted (HABE) group and the lower amount of blood extracted (LABE) group

Side effect	HABE group	LABE group	P value
Headache	1	4*	0.336
Hijama-site pruritus	1	3*	0.602
Dizziness	0	0	1.000
Tired and sleepy	0	3	0.228
Nausea	1	0	0.467
Pain at hijama site	1	1	1.000
Same-day insomnia	0	0	1.000
Vomiting	0	0	1.000
Bullae formation	0	0	1.000

* 1 missing data.

When comparing the baseline BP measurements with the BP readings at 4 and 8 weeks of follow-up, a significant difference was noted within the hijama group, although similar results were also found within the control group. However, we believe that the BP reduction in the control group may be due to the short follow-up intervals — this may have made the participants more conscious of their BP, and may have led them to improve their diet and lifestyle, possibly positively influencing their BP measurements. However, these reductions were not the result of changes in anti-hypertensive medication types or compliance, as these factors were monitored throughout the study.

An uncontrolled observational study in China measured the BP outcomes of patients immediately after they underwent hijama sessions, and the values were compared with the baseline BP readings^[7]. The authors reported a significant difference in the readings, similar to that in the present study; however, we do not believe that this observation is clinically relevant because if hijama does not yield a relatively long-lasting BP reduction, it would not be applicable as a treatment as it is unrealistic to undergo the procedure daily.

The days for hijama procedures in the present study (days 17, 19, and 21 of the lunar month) were chosen according to Islamic literature. Some evidence in the published literature has indicated some relation between the lunar phase and blood pressure^[20]. In our previous pilot study, we did not choose specific days for conducting hijama sessions, and we did not observe any positive effect on BP^[16]. However, the identification of specific days for performing the hijama procedure requires further research, particularly considering that this variable has not been reported in previous hypertension studies.

Although we assessed many factors that could have affected the hijama procedure outcomes, the amount of

blood collected during the hijama session was the only one that showed a positive effect on the BP. As mentioned earlier, hijama patients in the HABE group had better BP outcomes than those in the LABE group. In particular, these groups showed significantly different SBPs and DBPs. However, as this measurement was not prospectively planned, the amount of blood was not accurately measured. Nevertheless, this is an important point that has not been described in previous studies. We believe that a greater number of incisions at each hijama site — 10 to 15 incisions — might yield more blood collection and consequently produce better results. Based on our results, we would not recommend undergoing 3 consecutive sessions, each spaced a day apart, because there was no BP difference between those who did one, two, or three sessions. One session might be enough to achieve the required result. This is an area that needs further research.

The BP-lowering mechanism of hijama is unknown. One hypothesized mechanism of action is the “Taibah Theory”, which states that hijama drains interstitial fluid, excess intravascular fluid, and noxious metabolic substances. The theory also hypothesizes that hijama stimulates endogenous nitric oxide production and excretion of accumulated vasoactive substances and free radicals, which may cause reduced BP measurements. All these effects are beneficial for treating hypertension, according to the theory^[21].

In the present study, hijama was demonstrated to be a generally safe and well-tolerated procedure. The most common immediate side effect was headache, with other less frequent side effects including pruritus, post-procedural sleepiness, dizziness, nausea, and insomnia; only one patient experienced pain at the cupping site. One patient experienced hypotension and vomiting after her first hijama session, immediately after seeing blood accidentally spilled from the collection cup; this may have been a vaso-vagal effect. She was stabilized before she left the clinic and her blood pressure returned to normal within a few minutes. That patient did not experience similar reactions following the subsequent two hijama sessions. The only late side effect was the presence of mild hyperpigmented scars that persisted 8 weeks after treatment in 10 of the 36 participants who completed the follow-up visits. Typically, these scars gradually disappeared over time, but this was not confirmed in this study. These side effects, compared with those associated with anti-hypertension medications, are considered mild^[4]. In addition, the HABE group did not experience additional side effects.

The present study has several positive factors. One such factor is its originality, since studies describing the effect of wet-cupping on hypertension are very rare. Another positive aspect is the study participants’ high follow-up rates. Only three participants (3.75%) were lost to follow-up at 4 weeks and seven (8.75%) at 8 weeks. This study



also involved a larger sample size than previous studies, giving it greater statistical power. Finally, we followed patients for a relatively long period, which made it possible to track the effects of hijama on BP over a long period of time.

This study's most important limitation, and that of all wet-cupping studies performed to date, is the inability to blind the study. This is due to the absence of a well-developed sham wet-cupping method. Although wet-cupping might induce a placebo effect, BP is an objective outcome that is unlikely to have a significant placebo effect. Another limitation is that the timing of the 4-week follow-up appointment was not accurate for all participants in both groups. This was largely overcome at the 8-week follow-up appointment.

5 Conclusion

Wet-cupping therapy effectively reduced SBP in hypertensive patients for up to 4 weeks, without any serious side effects. We recommend the use of this complementary treatment, in conjunction with anti-hypertension medications, to treat hypertension. Additional studies are also needed to investigate the efficacy of wet-cupping alone, without any concomitant anti-hypertension medications. Moreover, additional research on the effect of the number of incisions and the amount of blood collected during the hijama procedure is needed. We also recommend the development of a sham wet-cupping technique to aid future studies.

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7 Conflict of interest disclosure

The authors report the absence of any conflicts of interest with the funding organizations.

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Submission Guide

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• Research Article

Ultra-highly diluted plant extracts of *Hydrastis canadensis* and *Marsdenia condurango* induce epigenetic modifications and alter gene expression profiles in HeLa cells *in vitro*

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ABSTRACT

OBJECTIVE: Methylation-specific epigenetic process and gene expression profiles of HeLa cells treated with ultra-high dilutions (HDs) of two plant extracts, *Hydrastis canadensis* (HC-30) and *Marsdenia condurango* (Condu-30), diluted 10⁶⁰ times, were analyzed against placebo 30C (PI-30) for alterations in gene profiles linked to epigenetic modifications.

METHODS: Separate groups of cells were subjected to treatment of Condu-30, HC-30, and PI-30 prepared by serial dilutions and succussions. Global microarray data recorded on Affymetrix platform, using 25-mer probes were provided by iLifeDiscoveries, India. Slides were scanned with 3000 7G microarray scanner and raw data sets were extracted from Cel (raw intensity) files. Analyses of global microarray data profile, differential gene expression, fold change and clusters were made using GeneSpring GX12.5 software and standard normalization procedure. Before microarray study, concentration of RNA (ng/μL), RIN value and rRNA ratio for all the samples were analysed by Agilent Bioanalyzer 2100. Reverse transcriptase polymerase chain reaction (RT-PCR) and quantitative RT-PCR were done for analyzing SMAD-4 expression. Fluorescence-activated cell sorting study was further made to elucidate fate of cells at divisional stages. Methylation-specific restriction enzyme assay was conducted for ascertaining methylation status of DNA at specific sites.

RESULTS: HDs of HC-30 and Condu-30 differentially altered methylation in specific regions of DNA and expression profiles of certain genes linked to carcinogenesis, as compared to PI-30. Two separate cut sites were found in genomic DNA of untreated and placebo-treated HeLa cells when digested with McrBC, compared to a single cut observed in Condu-30-treated genomic DNA. SMAD-4 gene expression validated the expression pattern observed in microarray profile. Methylation-specific restriction enzyme assay elucidated differential epigenetic modifications in drug-treated and control cells.

CONCLUSION: HDs triggered epigenetic modifications and alterations in microarray gene expression profiles of many genes associated with carcinogenesis in HeLa cells *in vitro*.

Keywords: plant extracts; homeopathy; reactive oxygen species; apoptosis; gene expression; epigenesis, genetic; Smad4 protein

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1 Introduction

In the development of pharmacological drugs, some of the most common barriers include poor pharmacokinetics, insufficient therapeutic activity, drug toxicity, and poor bioavailability^[1]. Given these issues, complementary and alternative medicines (CAMs), which often use plant extracts and other natural products that are relatively less cytotoxic, have gained increasing popularity. Various scientific methods are now being applied to learn about their efficacy, proper dosage, and mechanisms of action. Homeopathy, a popular branch of CAM, uses both crude and ultra-highly diluted (potentized) forms of remedies. Homeopathy is often criticized for its failure to explain the mechanism of action of the ultra-high dilutions (HDs) and therefore needs closer examination with a modern scientific approach^[2]. An exhaustive survey in Britain suggests that 70% of all oncology departments employ at least one form of CAM treatment in cancer care^[3]. More recently, the National Center for Complementary and Alternative Medicine (NCCAM), under the National Institutes of Health (NIH), USA, approved homeopathy as an alternative medicinal approach under the regulation of Food, Drug and Cosmetic Act (FDCA)^[4]. However, the dearth of information on homeopathy's scientific mechanisms of action makes its acceptance rather difficult. In recent years, the documentation of the existence of nano-particles^[5,6] of the original drug substance(s) in some highly diluted remedies used in homeopathy has given a new impetus for conducting more rigorous research utilizing state-of-the-art techniques^[7-9].

Other studies have used *in vitro* and *in vivo* conditions in models like *Escherichia coli*, yeast, bacteriophage and plant-based experimental models to understand exact molecular mechanisms of highly diluted homeopathic remedies^[10-16]. Human tumor-derived cell line models used in cancer therapeutics programs are effective for the evaluation of potential new therapeutic agents. These cell line-based models are integrated with efforts to identify biomarkers of cancer progression and mechanisms of drug action^[17].

Human cervical cancer cell line HeLa belongs to HPV-18-positive cell line, which carries approximately 10–50 viral integrated copies^[18]. This is an ideal model to study genetic and epigenetic modifications by therapeutic agents. The epigenetic phenomenon is defined as a heritable reversible phenotype, resulting from changes in a chromosome without alteration in the DNA sequence. DNA methylation, histone modifications, nucleosome positioning, micro-RNAs and non-coding RNAs are the hallmark traits of epigenetics^[8,9,19].

Previously, the notable hypotheses put forth to explain the efficacy of homeopathic remedies include molecular

imprints or memory of water, similia principle, hormesis, nano-silicon principle, electromagnetic transfer through DNA wave principle, thermo luminescence, and gene regulatory and epigenetic hypotheses^[9,11,20-26]. Of these, the gene regulatory hypothesis of Khuda-Bukhsh^[11] is gradually gaining wide attention for its ability to explain the biological action of HDs in all living organisms, from prokaryotes through human beings; this hypothesis is being studied at the molecular level.

This study has been designed to examine the cervical cancer HeLa cell line, a suitable *in-vitro* model, to get insight into whether the HDs of two plant extracts, *Hydrastis canadensis* (HC-30) and *Marsdenia condurango* (Condu-30), can manifest any demonstrable changes in the global microarray profiles of HeLa cells by induction through epigenetic alterations. We also conducted experiments on functional validation of SMAD4 biomarker in cancer and methylation specific restriction enzyme (RE) digestion in order to make differential gene expression analysis of two HDs generally used against cancer, namely, HC-30 and Condu-30, versus placebo 30C (PI-30), the vehicle of the HDs, and if epigenetic alteration is one of the main pathways for bringing about necessary changes in gene expression profiles, to elucidate underlying mechanisms of action of the highly diluted homeopathic remedies at the molecular level.

2 Materials and methods

2.1 Chemicals and reagents

Dulbecco's modified Eagle medium (DMEM), fetal bovine serum (FBS), trypsin and ethylene diamine tetra-acetic acid (EDTA) were purchased from Gibco BRL (Carlsbad, CA, USA). Penicillin-streptomycin-amphotericin (PSA) antibiotic and Hipur A RNA-Xpress reagent were obtained from Himedia (Mumbai, India). Tissue culture plastic wares were obtained from Tarsons (Kolkata, India). Propidium iodide (PI) was purchased from Sigma Chemical Co. (St Louis, USA). Oligonucleotide primers were obtained from Imperial Life Sciences, India. Agarose was procured from Lonza, USA. Taq DNA polymerase, deoxynucleoside triphosphates (dNTPs) and reverse transcriptase enzymes were procured from Chromous Biotech (Bangalore, India). Methylation-specific restriction enzyme McrBC was procured from New England Biolabs, USA. All other chemicals used were procured either from Sigma, USA or from Merck, Germany, if not mentioned otherwise.

2.2 Preparation of HC-30, Condu-30 and PI-30

Crude ethanolic root extract of *Hydrastis canadensis* and *Marsdenia condurango* were dynamized to the 30th potency by following the standard serial dilution and succussion method as advocated in the European

Homeopathic Pharmacopeia (<http://www.echamp.eu/news/newsletter/newsletter-archive/2013/september/homeopathy-in-the-european-pharmacopoeia.html>; see Khuda Bukhsh^[27] for further detail by Boiron Laboratories (Lyon, France)). In brief, 1 mL of the crude ethanolic plant extract is mixed with 99 mL of 70% ethanol and given 10 mechanical jerks (succussions) of equal magnitude and the potency 1 is obtained. Again 1 mL of potency 1 is mixed with 99 mL of 70% ethanol and given 10 jerks to produce the potency 2 and so on. We procured both the HDs (diluted 10^{-60}), HC-30 and Condu-30, as well as the PI-30, in which the same stock of ethanol as in drugs was used, from Boiron Laboratories, Lyon, France.

2.3 Cell culture and treatment

HeLa cells were obtained from National Centre for Cell Science (NCCS), Pune, India. Cells were routinely maintained in DMEM supplemented with 10% FBS and 1% antibiotic at 37 °C in a humidified incubator containing 5% CO₂. Cells were treated with 4% (v/v) of either HC-30, Condu-30 or PI-30 and incubated for 48 h in CO₂ incubator. Cells without any treatment were considered as negative control.

2.4 Microarray experiment

Separate groups of cells were subjected to the treatment of Condu-30, HC-30, and PI-30. Cells were sent to iLifeDiscoveries, Gurgaon, India for providing us global microarray data conducted on Affymetrix platform, using 25-mer probes. The total number of probes detected for the experiment was 49 395; hybridization was done at 45 °C for 16 h at 60 × g.

Slides were scanned with 3000 7G microarray scanner and raw data sets were extracted from the Cel (raw intensity) files. Microarray data analysis, differential gene expression analysis, fold change analysis and cluster analysis were performed using GeneSpring GX12.5 software.

2.5 Experimental grouping

For microarray gene expression study, samples of untreated HeLa cells, and HC-30-, Condu-30- and PI-30-treated series were termed as control, SET I, SET II and SET III respectively. All sets were taken in triplicates.

2.6 RNA quality control before microarray experiment

Before the microarray gene expression study, concentration of RNA (ng/μL), RIN value and rRNA ratio for all the samples were analysed by Agilent Bioanalyzer 2100.

2.7 Data pre-processing and normalization

All the original microarray data (CEL files) for the experiment were pre-processed using robust multichip average (RMA) algorithm that consists of three steps: a background adjustment, quantile normalization and finally summarization. All above procedures were done by selecting RMA algorithm in GeneSpring GX12.5. Genes of low-intensity information content in each data

set were filtered as follows: the probes of intensities less than the 10.0 percentile in the raw data were excluded from the analysis. The step used in pre-processing and normalization is given below.

2.7.1 Raw signal values

The term “raw” signal values refer to the linear data after thresholding and summarization. Summarization is performed by computing the geometric mean.

2.7.2 Normalized value

“Normalized” value is the value generated after log transformation and normalization (scale) and baseline transformation.

2.7.3 Treatment of control probes

The control probes were included while performing normalization. However, there should be an exact match between the control probes in the technology and the sample for the probes to be utilized.

2.7.4 Sequence of events

The sequence of events involved in the processing of the data files was: thresholding > summarization (summarization is performed by computing the geometric mean) > log transformation > normalization > baseline transformation.

2.7.5 Baseline to median of all samples

For each probe the median of the log summarized values from all the samples was calculated and subtracted from each of the samples.

2.8 Box whisker and profile plot

The box-whisker plot presents the normalized microarray expression data visualization summary. Further data are also distributed on conditions in the active interpretation with respect to the active entity or gene list in the experiment. The box-whisker plots are created between normalized intensity values and all probes.

The profile plot describes the summary of overall expression patterns of microarray experimental data.

2.9 Principle component analysis plot

After data normalization, quality control (QC) on samples was performed to remove the unreliable data from further analysis. The QC results are shown in the form of principle component analysis (PCA) in a 3D scatter plot. The scores are used to check data quality. It shows one point per array and is colored by the experiment factors or conditions. This allows viewing of separations between groups of replicates. Ideally, replicates within a group should cluster together but be separate from arrays in other groups.

2.10 Hybridization and correlation plot

Hybridization quality was checked by comparison with “hybridization control”. Hybridization controls were composed of a mixture of biotin-labelled cRNA transcripts of bioB, bioC and bioD, and prepared in staggered concentrations (1.5, 5, 25, and 100 parts per million (PPM), respectively). This mixture was spiked into the hybridization cocktail.

BioB was at the level of assay sensitivity and should be considered at least “50% present” at the time. BioC, bioD and cre must be “present” all of the time and must appear in increasing concentrations.

2.11 Gene expression analysis

A total number of 1 345 genes were found to be oppositely expressed in SET I (HC-30) *vs.* control and SET III (PI-30 or placebo) *vs.* control. Number of genes with ≥ 1.5 -fold differential expression was found to be 23. The 1.5-fold change was used because during the fold change analysis, 1 out of 3 of the compared conditions was considered valid. This means if any gene showed up-or down-regulation ≥ 1.5 in any 1 out of the 3 conditions, this would be included in the fold change results. This is important for the gene regulation pattern identification. If one gene is expressed below 1.5 in two conditions and the same gene has expressed more than 1.5 fold in 1 condition, the gene could be considered as showing a change in expression. If we selected the condition 3 out 3 then we would filter out only those genes that have consistence in fold change ≥ 1.5 in all three compared conditions. So fold change 1.5 in 1 out of 3 was deemed optimal. This parameter was also suggested for time series, dose and drug response microarray experiments. How this particular analysis was carried out includes the chance that we may have missed identifying some important gene information from our data, because of the possibility that some gene expressions were instantly decreased/increased in any conditions (less than 1.5 fold) after drug treatment, and these were not counted.

A total number of 650 genes were identified as oppositely expressed between SET II (Condu-30) *vs.* control and SET III (ethanol-30 or placebo) *vs.* control. The number of genes with ≥ 1.5 -fold differential expression was found to be 12.

A total number of 1 182 genes were identified to be oppositely expressed between SET I (HC-30) *vs.* control and SET II (Condu-30) *vs.* control. The number of genes with ≥ 1.5 -fold differential expression was found to be 36.

2.12 Hierarchical clustering

Cluster analysis was performed for the identification of similar type of experiments or co-expressed gene sets across the sample for the differentially expressed genes. Clustering can group the genes having similar type of expression. Unfortunately, because of the limited number of experiments run (largely due to prohibitive costs), the

cluster analysis did not yield significant results. But gene alteration using heatmap image of hierarchical clustering was more revealing, showing more clear differential expression of genes for individual experiment sets.

Hierarchical clustering is one of the simplest and widely used unsupervised clustering techniques for the analysis of gene expression data. The method follows an agglomerative approach, where the most similar expression profiles are joined together to form a group. These profiles are further joined in a tree structure, until all data form a single group. The dendrogram is the most intuitive view of results of this clustering method. There are several important parameters, which control the order of merging entities and sub-clusters in the dendrogram. The most important of these is the linkage rule. After two most similar entities (clusters) are clubbed together, this group is treated as a single entity and its distances from the remaining groups (or entities) have to be recalculated.

2.13 Qualitative reverse transcriptase-polymerase chain reaction and quantitative reverse transcriptase-polymerase chain reaction analysis of SMAD4

For quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR) analysis the method described by Saha and Khuda-Bukhsh^[28] was followed. Equal amounts of total RNA extracted with RNA expression reagent (Himedia, Mumbai, India) were reverse-transcribed using random hexamer primer and then subjected to PCR with enzymes and reagents of the reverse transcription system using Techne PCR system (Staffordshire, UK). Sequences of primers used in the study are given in Table 1.

Quantitative measure of SMAD4 gene expression analysis was further done by qRT-PCR on ABI 7900HT by relative quantification using the comparative C_T method. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was chosen as an internal control for normalization. For all the samples, the median C_T values for the target genes and GAPDH were taken and the expression of target genes was normalized with that of GAPDH. The primer used in the assay is of the same sequence as used in qualitative RT-PCR study.

2.14 Epigenetic study by RE digestion assay

In order to determine methylation status of genomic DNA of HeLa cells in HC-30-, Condu-30-and PI-30-treated and untreated series, the RE digestion method was employed. First, DNA samples were prepared for RE study by following the rapid isolation method^[29] consciously to

Table 1 Primer sequences for qualitative RT-PCR and quantitative RT-PCR analyses of SMAD4

Primer	Forward 5'–3'	Reverse 5'–3'
SMAD4	TCCTGTGGCTTCCACAAGTC	TCCAGGTGGTAGTGCTGTTATG
GAPDH	CAGCCTCAAGATCATCAGCA	TGTGGTCATGAGTCCTTCCA

RT-PCR: reverse transcriptase-polymerase chain reaction; GAPDH: glyceraldehyde-3-phosphate dehydrogenase.

avoid the use of phenol-chloroform. In brief, cells were harvested and dissolved in ice-cold lysis buffer containing 10 mmol/L Tris-HCl pH 8.0, 1 mmol/L EDTA pH 8.0 and 0.1% SDS with proteinase K (20 mg/mL) followed by incubation at 55 °C for 3 h. The digest was then incubated further at 37 °C for 1 h with RNase (4 mg/mL). Next, potassium acetate solution (60 mL of 5 mol/L potassium acetate, 11.5 mL of glacial acetic acid and 28.5 mL of water) was added and mixed by vortexing. Protein-SDS complex was precipitated by centrifugation at $8\,000 \times g$ for 15 min. The supernatant was transferred to a fresh tube containing isopropanol. It was then mixed and again centrifuged at $8\,000 \times g$ for 15 min. The DNA pellet was washed with 70% ethanol. The semi-dried DNA was dissolved in TE buffer at pH 7.0. Further quality checking was carried out by measuring optical density (OD) at 260/280 nm (ratio maintained at 1.6–1.8) to confirm that DNA was free of protein contamination. After confirmation of quality, DNA was used for further experiments. Methylation-dependent enzyme MspI (New England Biolabs) was used in the study. Genomic DNA (1.0 µg) was digested with 10 units of RE, in 50 µL total reaction mixtures supplemented with reaction buffers and accessory reagents for overnight at 37 °C. The digested DNAs were electrophoresed on 1.5% agarose gel followed by ethylene bromide staining to observe differential band patterns, if any.

2.15 DNA content or ploidy analysis in terms of cell cycle distribution by flow cytometry

HeLa cells were grown at an equal density of 2×10^6 cells in 90 mm culture plate and treated with HC-30, Condu-30 and PI-30. The cells were harvested and fixed in ice-chilled 80% ethanol with constant vortexing and stored at -20 °C for future use. For flow cytometric analysis, cells were separated from the fixative by centrifugation and washed in staining buffer (1× phosphate buffered saline, 2% FBS, 0.1% sodium azide at pH 7.1–7.4). RNase (100 µg/mL) was added and the cells were incubated at 37 °C for 2 h. After incubation 50 µg/ml PI was added to the material and kept for 30 min at 4 °C. Cell cycle analysis was performed using the BD FACS Verse™ system and data were analyzed using the BD FACS Diva™ software. For each sample equal numbers of cells (10 000) were counted. Cell cycle distribution analysis was done by gating the cell population^[30].

2.16 Blinding

The observer was kept “blinded” during the main part of observation, after which the codes were deciphered for data analysis.

3 Results

3.1 RNA quality analysis data

All samples showed RIN values above 6.0, considered

highly satisfactory for microarray analysis. These samples were therefore further processed for gene expression microarray experiment.

3.2 Box-whisker and profile plot data

The total number of probe sets detected for the experiment was 49 395. After data pre-processing, normalization and quality control on data, 40 678 probe sets remained out of 49 395. Baseline transformation was performed by taking median of all probe sets. Transformation was shifted from 20% to 75% for raw intensity data in GeneSpring tool on the basis of median value. The box-whisker plots were created between normalized intensity values and all probes. The box-whisker plots showed the median in the middle of the box, the 25th quartile and the 75th quartile. Box whisker plots had been generated for all samples for expression pattern visualization. The same replicate experiment set had similar expression pattern in the whisker plot (Figures 1A and 1B). All experiment entities (probe sets and genes) were represented separately in the form of line graphs. The experiment name is represented at X-axis, and Y-axis shows the normalized intensity value for the experiment (Figure 1C).

3.3 PCA plot

The PCA components, represented in the X, Y and Z axes, are numbered 1, 2 and 3, according to their decreasing significance (Figure 2).

3.4 Hybridization and correlation plot

The X-axis in this graph represents the controls and the Y-axis, the log of the normalized signal values. The correlation plot shows the correlation analysis across arrays. It finds the correlation coefficient for each pair of arrays and then displays them in a textual form as a table as well as in the form of a heatmap. The correlation coefficient is calculated using pearson correlation coefficient (Figures 3A and 3B).

3.5 Cluster analysis

In the current study hcl (average linkage) was performed on “conditions” and “genes” to explore the co-expression or co-regulation of genes in three groups. First cluster analysis performed on all experiment sets (SET I, SET II, SET-III and control) tried to explore biological similarity among experiment sets. The hcl expression image was presented in the form of a dendrogram. The expression image tree was further characterized on the basis of color. The red color shows over-expression, blue color shows under-expression and yellow color shows normal expression of genes. In the condition tree, experiments having similar expression profiles clustered adjacently in the tree. The genes having similar type of expression profiles also clustered adjacently or on the same tree node (Figure 4).

3.6 Functional validation of SMAD4 gene

There was down-regulation observed in PI-30-, HC-30- and Condu-30-treated series compared to the untreated

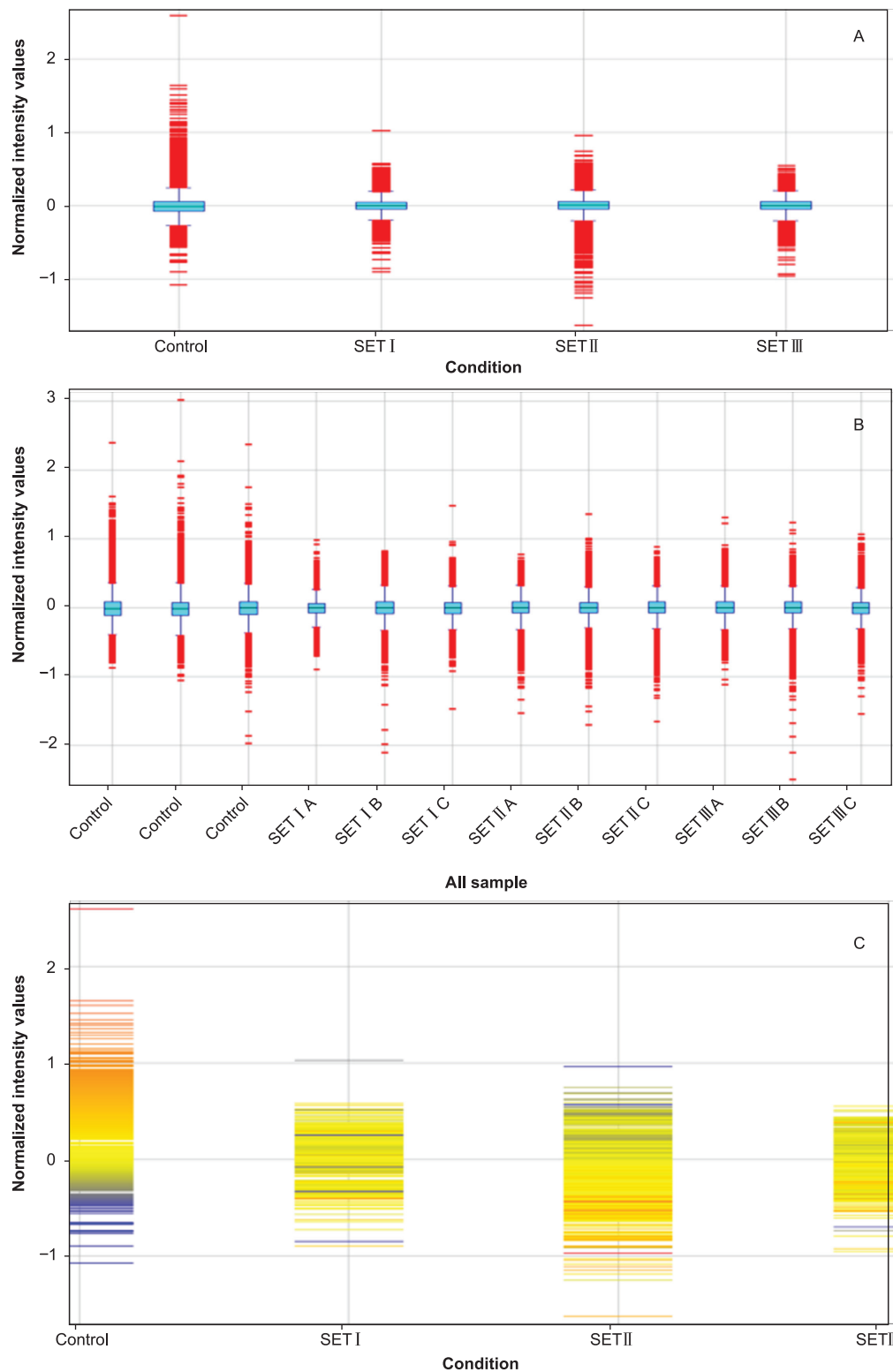


Figure 1 Box-whisker and profile plot

(A) Box-whisker plot of different groups. (B) Box-whisker plot of individuals. Data are robust multichip average normalized from all experiment sets (data align well from baseline to median, and microarray experiment was successful). It is also clear from the image that same replicate experiment has closely related expression pattern. (C) The profile plot shows over all expression patterns of genes. The Y-axis shows normalized ratio and X-axis shows experiment set name. The normalized expression value >0 shows over-expressed genes and <0 shows under-expressed genes.

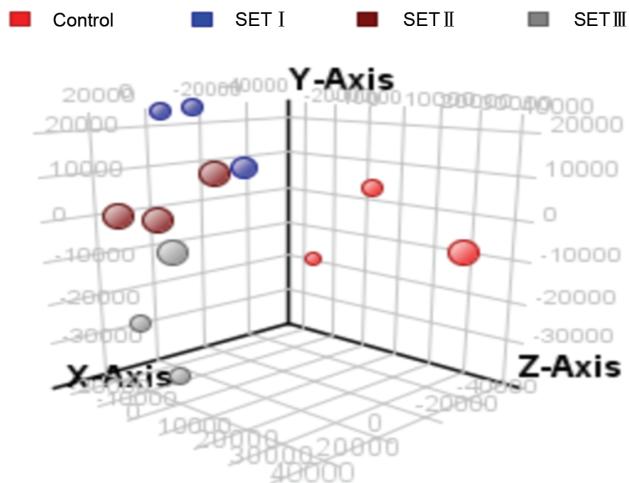


Figure 2 Principle component analysis plot

control. Fold change values were as follows: for placebo it was 1.76, for HC-30C the fold change was 3.867 and for Condu-30C it was 4.72 (Figures 5A and 5B).

3.7 Alteration in methylation status: an event of epigenetic alteration

Alteration of methylation-status of DNA was analyzed using methylation-dependent enzyme McrBC digestion. There appeared to be two separate cut sites in genomic DNA obtained from untreated and placebo-treated HeLa cells when digested with McrBC, in contrast to a single cut observed in DNA of Condu-30-treated genomic DNA after proper normalization (Figure 5C).

Changes observed were marginal when DNA was digested with HC-30 used for the methylation-dependent enzyme McrBC digestion (Figure 5C).

3.8 Cell cycle distribution analysis

There was increased accumulation of cells at G_0/G_1 population by 10.25% in HC-30-treated HeLa cells, compared to 6.62% in untreated HeLa cells and 4.59% in PI-30-treated HeLa cells (Figure 6). In case of HC-30-treated

HeLa cells the G_0/G_1 population was found to be 2.83%.

4 Discussion

Our previous study^[7] showed that the expression profiles of certain genes of HeLa cells treated with HC-30 and Condu-30, respectively, were significantly different from that of the PI-30-treated cells. Both the drugs and placebo differed in their ability to trigger gene responses, some of which were implicated in cancer. Thus, an analysis of data obtained in this global microarray study would be able to demonstrate whether the HDs can trigger gene responses in a cascade of reactions consistent with the hypothesis first proposed by Khuda-Bukhsh^[11,27]. Ideally, the microarray data would have been tested by making qualitative and quantitative analyses of expressions of many candidate genes, but due to financial and resource constraint, we could validate the microarray data by RT-PCR and qRT-PCR studies of only one important cancer-related candidate gene, SMAD4. Incidentally, Belavitte *et al*^[29] also obtained evidence through transcriptome analysis that HDs of *Gelsemium sempervirens* could alter expression profiles of genes in neurocytes. Very recently, Bigagli *et al*^[30] also demonstrated the effects of homeopathic *Apis mellifica* preparations on altered gene expression profiles of human prostate cells. The ability of HDs to trigger gene expression profiles is not limited to animal models. Marotti *et al*^[31] documented clear evidence of change in expression profiles of wheat seedlings in the plant kingdom following treatment with ultra-high dilutions of arsenic trioxide used as a homeopathic remedy against symptoms of arsenic poisoning. Thus substantial recent evidence on microarray analysis, which is an accepted modern tool of studying large scale gene expression profiles, clearly depicts that homeopathic remedies in ultra-high dilutions can trigger altered gene expressions presumably through epigenetic modifications, validating the “gene regulatory hypothesis” first advocated by Khuda-Bukhsh^[11,27].

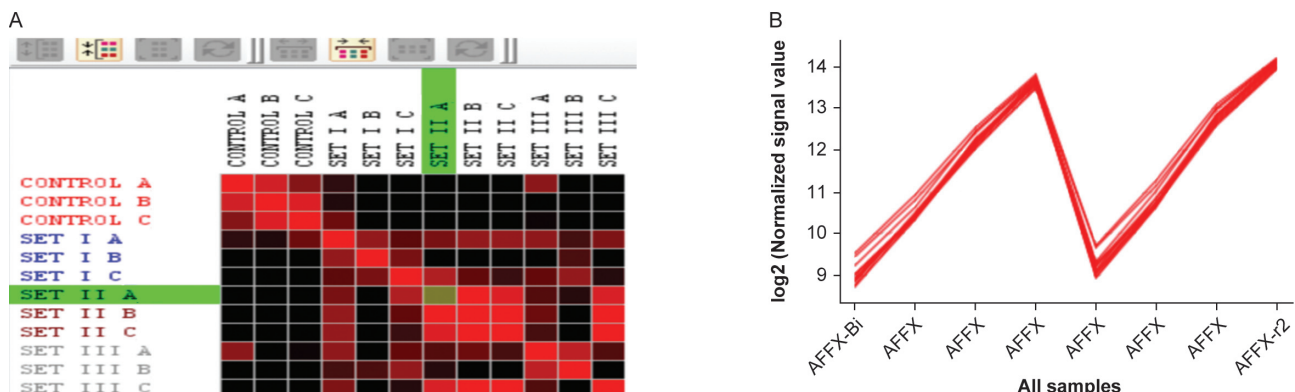


Figure 3 Hybridization and correlation plot

(A) Pair-wise correlation plot for each experiment; (B) Hybridization plot with control probes.

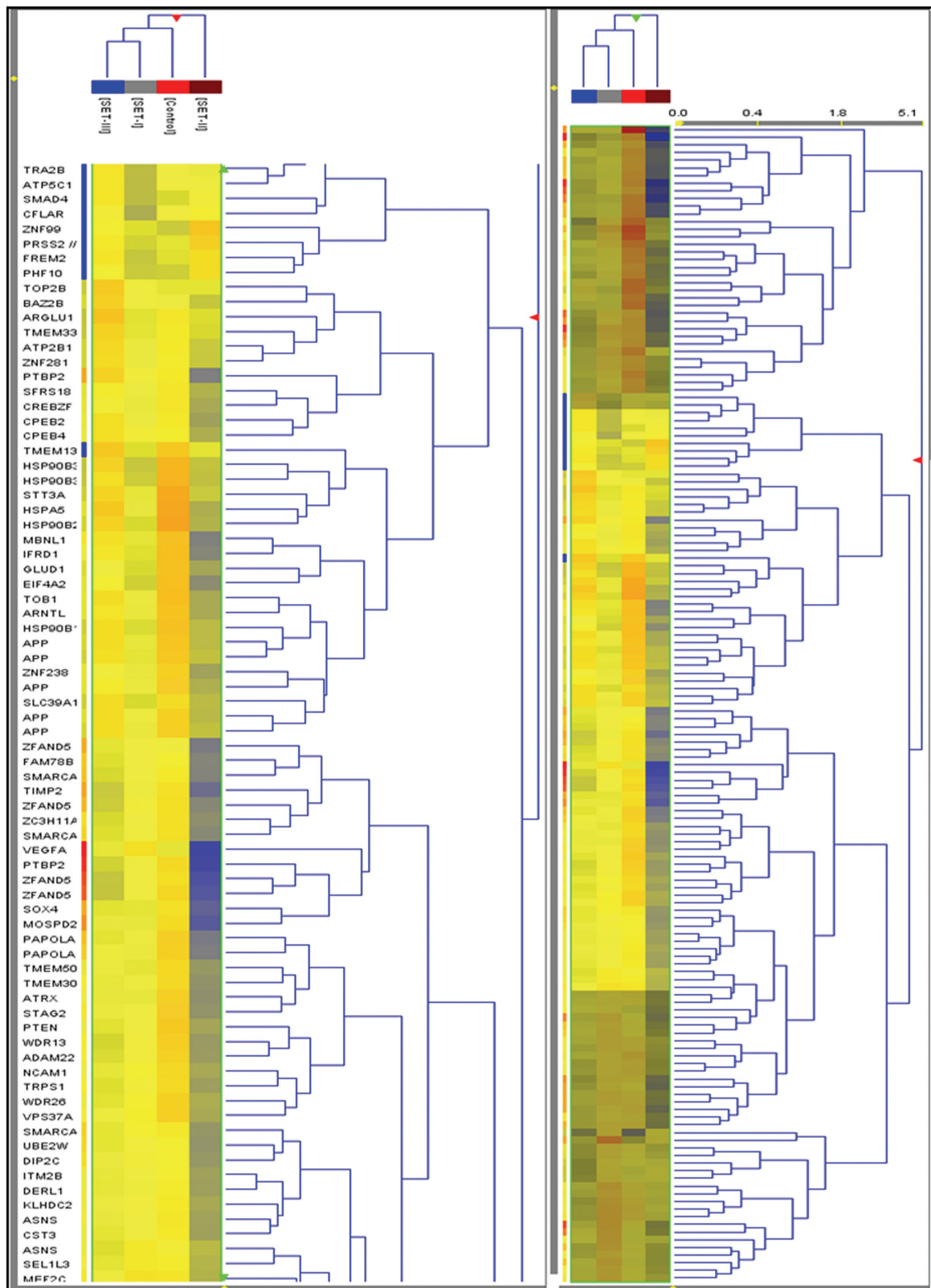


Figure 4 Cluster analysis

The snap shot of the heatmap image on experiment conditions and genes. The heatmap image was generated on the experiment conditions and classified on the basis of gene expression. Red color shows over-expressed genes (>0) and blue color shows under-expressed genes (<0).

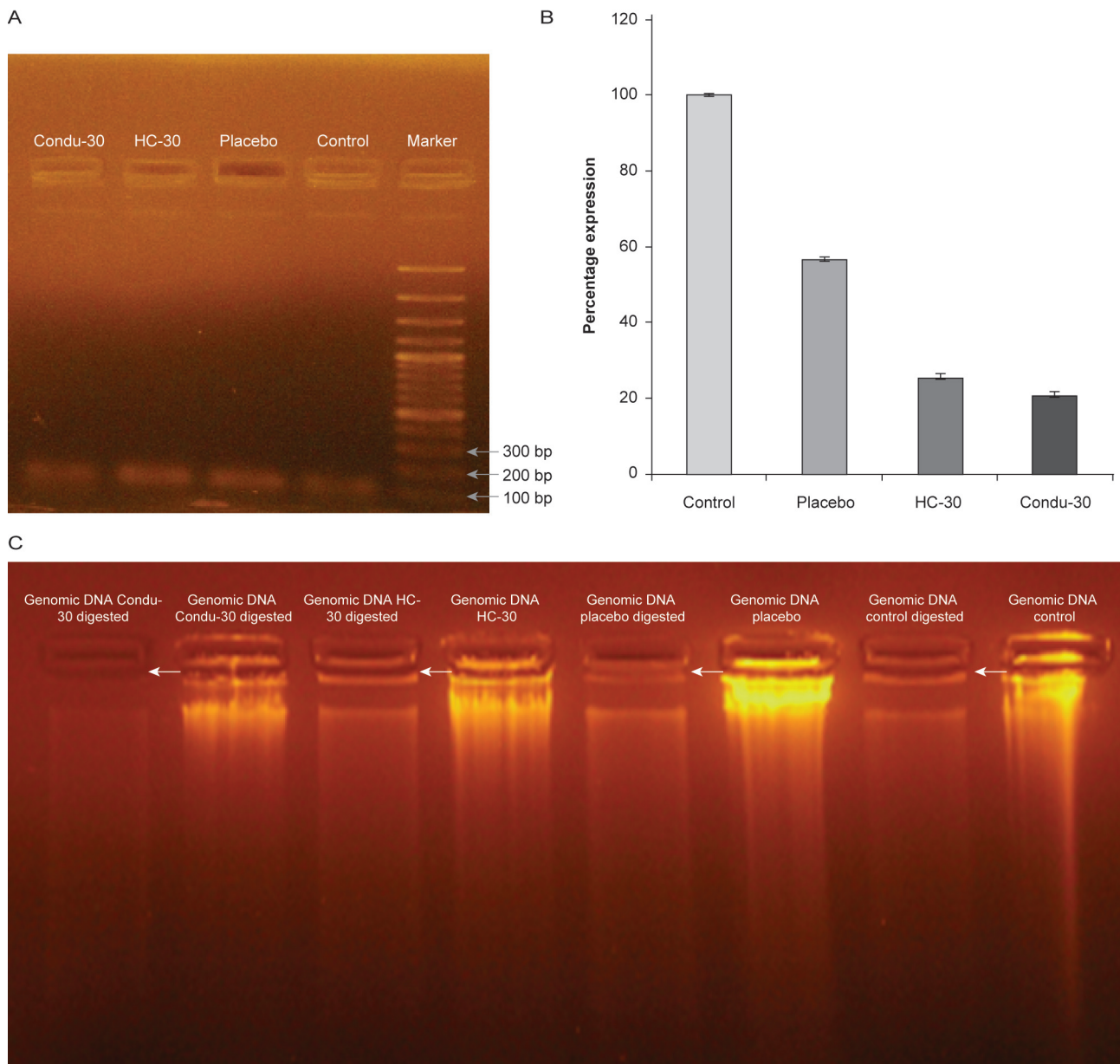


Figure 5 Functional validation of SMAD4 and methylation-dependent restriction enzyme digestion (A) Qualitative RT-PCR study of SMAD4; (B) Quantitative RT-PCR study of SMAD4; (C) Methylation-dependent MspI digestion of genomic DNA obtained from untreated, HC-30-, Condu-30- and placebo-treated HeLa cells. RT-PCR: reversed transcriptase-polymerase chain reaction.

Epigenetic modifications are a hallmark of cancer, and a large number of genes remain in modified state of expression in cancer cells. Our present study of alteration in methylation status of DNA by Condu-30 by MspI RE supports the previous findings obtained by Bishayee *et al*^[8]. Additionally, the results of the present study would further lend support to the gene regulatory hypothesis^[11,27,32] that can effectively be achieved by the epigenetic modification triggered by the HDs. Epigenetic modification is one way through which the expression of genes can be strongly

influenced and regulated as per need and condition of the organism; it is particularly useful at abnormal physiological states or in disease states, when the regulatory systems are often error-prone.

Our present study revealed the increase in G₀/G₁ population by HC-30-treated set compared against untreated control and placebo-treated HeLa cells. Increase in G₀/G₁ population is an indication of apoptosis induction. The present findings thus confirm the apoptosis-inducing ability of Condu-30 observed in HeLa cells and H460 lung

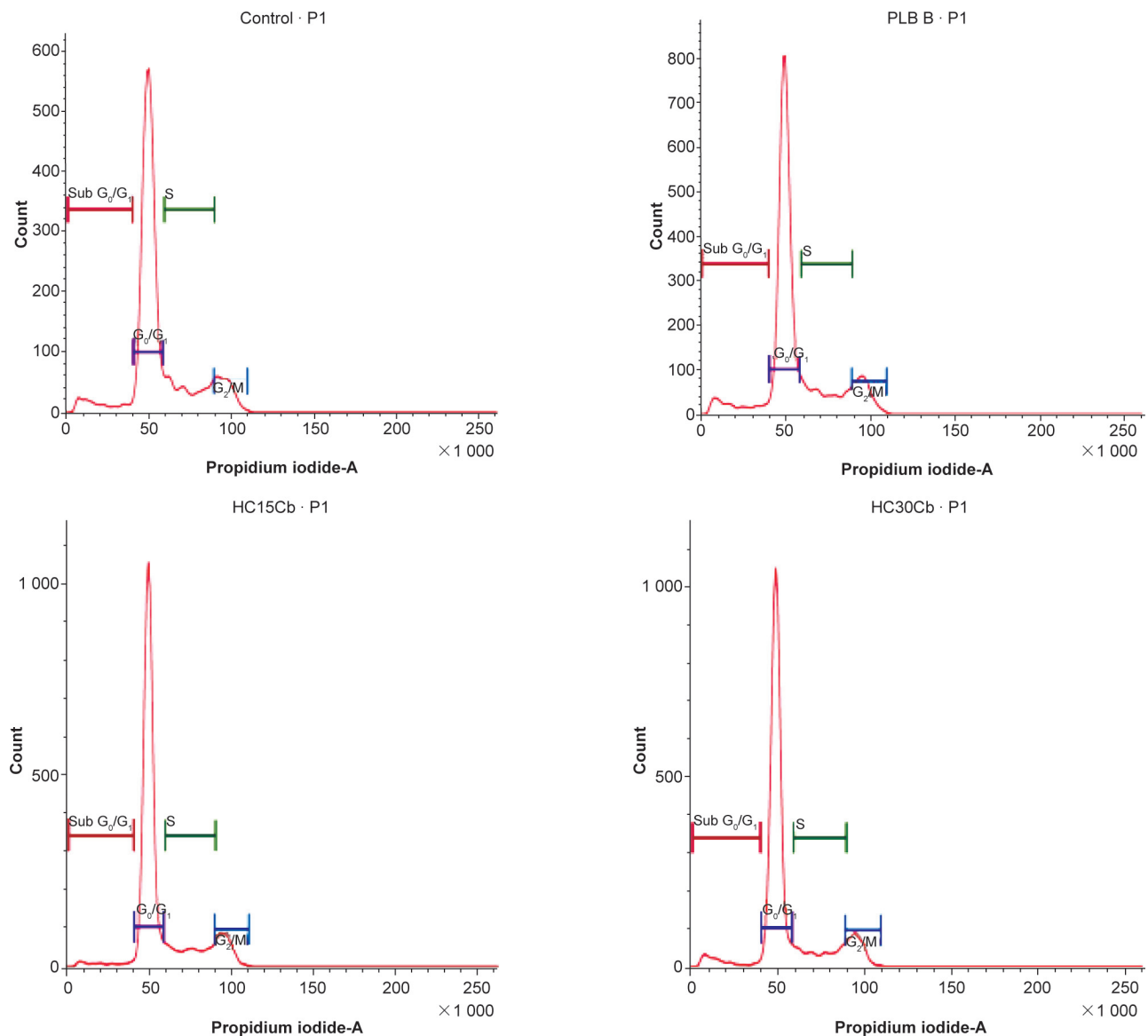


Figure 6 Cell cycle distribution study

cancer cells, also previously reported by our group^[8,9]. As cell cycle events are very much related to the structural and functional states of DNA, and the ability of the HDs to induce changes in DNA methylation status adds an important clue in the induction of apoptosis^[28,33], thus, various mechanisms of action shown by the HDs indicate the need for further in-depth studies to see if these phenomena can also be observed in other *in vitro* and *in vivo* experimentations. Our results give further credence for the gene regulatory hypothesis, which can explain the molecular mechanism of action of the HDs in a scientifically validated way. The ability of the HDs in modulation of various genes in the cell cultures indicates that they can have direct influence on the expression of relevant

genes, particularly when the HDs have been reported to elicit responses in unicellular organisms such as yeast^[34], bacteria and bacetriophages^[12–14].

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6 Competing interests

The authors declare that there are no competing interests.



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• Short Report

Attitudes of medical students toward the practice and teaching of integrative medicine

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ABSTRACT

The General Medical Council encourages the integration of complementary and alternative medicine (CAM) teaching into basic medical education. We wished to explore the attitudes of medical students to CAM and its inclusion in their undergraduate curriculum. Medical students were invited to complete the validated Integrative Medicine Attitude Questionnaire (IMAQ) and to state whether they considered it appropriate for them to learn about CAM in medical school. The questionnaire was completed by 308 students (65.8% response rate). CAM had been received by a majority of respondents and their families. Participants believed that doctors with knowledge of CAM provide better patient care and that it is desirable for physicians to exploit the placebo effect. Most students expressed the view that doctors should be able to answer patients' questions about herbal medicines. There was a belief that patients should be warned to avoid using supplements which have not undergone rigorous testing. Students who were current or previous users of CAM or whose family members used CAM had higher total IMAQ scores and openness subscale scores than those who did not report use of CAM. Two-hundred and nine (68%) students expressed a desire to study CAM as part of their medical curriculum. This study reveals a positive attitude towards a holistic approach to patient care which embraces CAM. Medical students believe that integrative medicine should be taught in medical school.

Keywords: attitude of health personnel; students, medical; complementary therapies; education

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1 Introduction

Integrative medicine (IM) is an approach to the practice of medicine that makes use of the best-available evidence, taking into account the whole person, including all aspects of lifestyle^[1]. IM includes orthodox approaches as well as complementary and alternative medicine (CAM) and encompasses a model focused on prevention, wellness, and healing^[2]. IM adopts a humanistic, partnership approach to care with an emphasis on providing the patient with hope, education and therapeutic approaches that match the individual's global perspective^[3].

CAM therapies may be based on substances, nutrition, manipulation, exercise, and mind-body interactions^[4]. They are not considered part of conventional medicine because of insufficient scientific evidence of their effectiveness. Despite this, use of CAM does not appear to be confined to any particular socioeconomic group, and is common in underserved populations^[5].

In the US, yearly visits to alternative practitioners had increased to 629 million visits by 1997, exceeding total visits to all primary care physicians^[5]. A later study showed that almost 4 out of 10 adults had used CAM therapy in the previous 12 months^[6]. An Australian study demonstrated

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that the estimated number of visits to CAM practitioners by adults in the 12-month period was almost identical to the estimated number of visits to medical practitioners^[7]. Reasons cited for the increase in popularity of alternative therapies include dissatisfaction with conventional health care, which is reported by patients as ineffectual, expensive, or overly focused on curing disease rather than maintaining good health^[7].

Currently, CAM is not well represented in undergraduate medical curricula in Europe. There is a need to develop successful strategies to evaluate both medical student and physician attitudes towards CAM and to incorporate information about CAM into already congested health professional curricula^[8]. This study was designed to explore the attitudes of medical students toward IM and to evaluate their level of interest toward the introduction of CAM into their curriculum. Students' attitudes toward CAM have been shown to be influenced by a number of factors including gender, age, race, and whether students had previously visited a CAM practitioner^[9-15].

2 Methods

2.1 Study design and data collection

The study was carried out using a web-based survey of medical students in five-year groups of our medical school. The students were sent an email inviting them to complete an anonymous electronic survey exploring their attitudes towards CAM. At the time these data were collected there was no CAM included in either the core or elective undergraduate medical curriculum of this institution.

2.2 IMAQ questionnaire

The questionnaire distributed was a modified version of the previously validated Integrative Medicine Attitude Questionnaire (IMAQ)^[10]. IMAQ is a 29-item, 7-point Likert scale-rated instrument. A total integrative medicine attitude score is created by summing the responses to each item. Sixteen of the questions were positive statements and thirteen were negative. A maximum score of 203 is possible using this instrument. A two-factor model was used based on the factor analysis which yielded Cronbach alpha coefficient values of 0.91 and 0.72, respectively: (1) openness to new ideas and paradigms; and (2) value of both introspection and relationship to the patient^[10]. Maximum possible scores for the openness factor and the value of introspection and relationship factors were 147 and 56, respectively.

The modified IMAQ questionnaire also collected further demographic data about the students' age, gender, race, whether they had used CAM previously or been cared for by a CAM practitioner, and whether any member of their family had ever used any CAM therapies. Results were expressed as medians with interquartile ranges and

a Kruskal-Wallis test was used to test for significant associations between selected variables.

3 Results

3.1 Demographics

A total of 308 out of 468 (response rate 65.8%) students completed the questionnaire, 57.5% of whom were female. Seventy-two percent ($n = 223$) of the respondents were of Irish nationality, with Malaysian students comprising the majority of the international cohort (22.7%, $n = 70$). Nearly two-thirds of students who responded reported family use of CAM (62%, $n = 191$), while fewer than half of students surveyed declared that they personally used CAM (42.5%, $n = 131$).

3.2 IMAQ scores

There were no significant differences between the students' particular year of study or nationality with total or subscale IMAQ scores (Table 1). There was a non-normal distribution of total IMAQ score, with a median score of 128 and interquartile range of 19. Participants scored higher in the "relationships" subscale (median = 43; 77% of maximum score) than the "openness" subscale (median = 90; 61% of maximum score). There was a statistically significant difference in the gender difference observed on the "openness" subscale (male median = 88, interquartile range 18; female median = 92, interquartile range 14.5; $P < 0.05$), but not on the "relationships" subscale (male median = 43; female median = 42).

Students who were current or previous users of CAM or whose family members used CAM had higher total IMAQ scores and "openness" subscale scores than those who did not report personal or family use of CAM. Two-hundred and nine (68%) students expressed a desire to study CAM as part of their medical curriculum. Figure 1 shows that students who had higher total IMAQ scores (median = 136.7) were more likely to express a desire to study CAM in their undergraduate medical curriculum than those who had lower total IMAQ scores (median = 123.7, $P < 0.05$).

3.3 Individual IMAQ item scores

Students were more positive than negative (*i.e.*, median item score ≥ 5) in relation to the following individual IMAQ items: knowledge of multiple medical systems; patient spirituality and healing; acupuncture in patients receiving chemotherapy; end of life care; placebo effect; healing in incurable diseases; physicians modelling balanced lifestyles; quality of life measures in research; personal change and growth of patients; innate healing of patients; patient-physician relationship; physicians striving to understand themselves; instilling hope in patients; patients' queries regarding botanical medicines; and nutritional counselling. Few items were rated more negatively than positively by the medical students (*i.e.*, median item score

Table 1 IMAQ scores presented by student gender, nationality, year of study, and use of CAM

Independent variable	Total IMAQ score	Openness subscale score	Relationships subscale score
Gender			
Male	131	88	43
Female	134	92*	42
Nationality			
Irish	134	91	43
Non-Irish	136	93	43
Year of study			
Foundation	133	90	43
Year 1	134	90	44
Year 2	133	90	43
Year 3	131	89	42
Year 6	134	92	42
Previous use of CAM			
Personal			
Yes	139*	91	43
No	129	88	43
Family			
Yes	136*	90	43
No	127	88	43

IMAQ scores are expressed as the nearest whole number. Only items marked with an asterisk achieved statistical significance by one-way analysis of variance ($P < 0.05$).

IMAQ: Integrative Medicine Attitude Questionnaire.

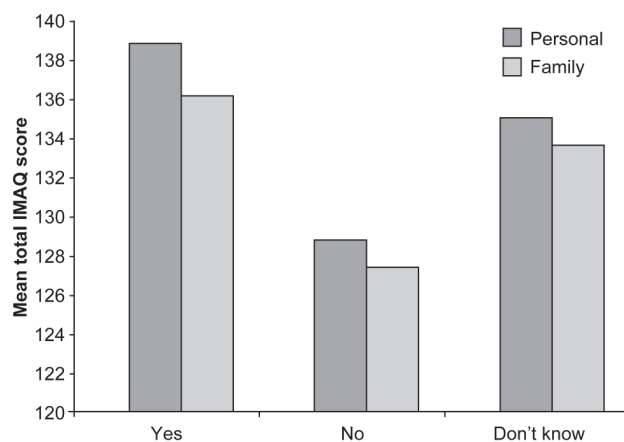


Figure 1 The relationship between student participants' personal and family use of CAM and their motivation to study CAM in medical school

CAM: complementary and alternative medicine; IMAQ: Integrative Medicine Attitude Questionnaire.

≤ 3): healing and pathological processes; physical healing; testing of herbs and supplements; and doctors' use of intuition.

4 Discussion

There has been a gradual increase in the reported use

of CAM by members of the public over the last few decades^[11]. This descriptive study examined the attitudes of undergraduate medical students towards the use of CAM, the relevance of IM, and their incorporation into the medical curriculum. The response rate was satisfactory and was representative of the approximate distribution of Irish and non-Irish medical students at our university. There was no difference in IMAQ scores between pre-clinical and clinical medical students or between Irish and international students. A previous study in the UK found that junior medical students were more positive toward CAM and its instruction than senior medical students^[12]. Schmidt and colleagues^[9] found a racial difference in their study of medical students' attitudes toward holism, with Caucasian students being more favourable towards it than Asian or black students. The authors concluded that Asian students choosing to study medicine may be more likely to reject traditional therapies associated with their native cultures in favour of Western allopathic medicine.

Levels of personal and family exposure to CAM were high in our study and positively correlated with total and openness subscale scores using the IMAQ questionnaire. Greiner and co-workers^[13] estimated CAM use among medical students at between 40% and 74%. Shankar and colleagues^[14] demonstrated a higher openness score among Nepalese medical students whose families used both CAM and allopathic therapies.

In the present study, female students scored higher on the openness subscale of the IMAQ questionnaire. Rees and co-workers^[15] found that students who expressed more positive views towards holistic patient care were more likely to be female and that their attitudes towards CAM became more positive over time. The majority of items on the IMAQ questionnaire were rated as more positive than negative by the medical students, indicating a favourable attitude towards the use of CAM and its integration into patient care.

Over two-thirds of students wished to see the introduction of IM into their medical studies, and special study modules were identified as the preferred means of introducing it to students. The UK General Medical Council advocates that medical graduates should show an awareness of the range of complementary therapies available to patients and the evidence for their use^[16]. CAM now features in most American medical school curricula^[17]. Providing students with a background in CAM may give them a better insight into their patients' motivation for using complementary therapies and will equip them to address their patients' questions regarding these treatments^[18]. We propose that medical school curriculum committees should give consideration to the integration of CAM into their spiral curricula at multiple levels, including in discussions on patient autonomy in ethics modules,

patient empowerment in discussion of health promotion approaches, and evaluating the medical literature in evidence-based medicine modules. Ideally CAM should appear as an adjunct to existing core material in the clinical phase of the curriculum, where evidence-based CAM therapies should be introduced to students in relation to common clinical problems.

Medical students believe that integrative medicine should be taught in medical school. While this is a cross-sectional study in a single site, the satisfactory response rate, and the mix of indigenous and international students of both genders adds to its generalisability. Future studies should evaluate the effectiveness of student-selected components as an educational tool to introduce medical students to IM and should compare their experiences with the approaches of medical schools which have embedded IM into their core curricula.

5 Acknowledgements

We are grateful for the statistical advice provided by Ms. Gloria Avalos, School of Medicine, National University of Ireland, Galway.

6 Competing interests

The authors state that they have no competing interests.

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• Study Protocol

Health effects of natural spring waters: A protocol for systematic reviews with a regional case example

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ABSTRACT

BACKGROUND: Spring water therapies have been used since at least 1550 BC. Despite the growing body of evidence supporting these therapies for a range of conditions, including musculoskeletal, dermatological, respiratory and cardiovascular conditions, they do not currently form part of mainstream healthcare in many countries. The protocol established in this paper aims to support systematic reviews that examine the health outcomes associated with human exposure to regional spring waters, using the Australia and New Zealand context as a case study.

METHODS/DESIGN: The protocol searches for studies in eight health/medical databases, searches three local health/medical journals, and includes forwards and backwards searching. Standard systematic review methods are used including: specifying pre-determined inclusion criteria and data management plans, appraising the studies for bias, and allocation to a hierarchy of evidence.

DISCUSSION: The protocol supports a review and comprehensive synthesis of the current evidence regarding the health effects of natural spring water, and can be adapted for reviews in other regions. From this evidence, recommendations regarding practice and future research can be made on the therapeutic role of spring water.

Keywords: hot springs; Australia; New Zealand; review; study protocol

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1 Background

Spring water has reportedly been used therapeutically since at least 1550 BC^[1]. Hippocrates of Kos reputedly used balneology to cure his patients^[2], and reported that “... that cold water warms, ... whilst warm water cools the body; ... that warm shower baths induce sleep, ... and

that cold water stimulates; ... he recommended cold water to assuage fever and pain”^[3]. Despite the widespread use of spring water as therapy, it was not until 1702 that more formal research into spring water therapies was conducted^[3].

In the region used as a case study in this paper, Australia and New Zealand, it is thought that hot springs have been

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used for healing by the Māori since 1300 AD^[4]. Since European settlement, the springs have been used and commercially developed starting in Australia from the 1850s^[5]. During the later nineteenth century and early twentieth century, spas in both countries were promoted by medical practitioners, with government support^[5,6]. At the time, treatment with spring water was a cheaper alternative to conventional treatments, and applied via stream, baths, douches or by ingestion^[6].

Although the spa industry fell out of favour internationally from the 1950s, there was a resurgence of interest in the 1980s, and the industry has continued to thrive, resulting in recent investment into springs such as Moree, Peninsula Springs and Hepburn Springs in the State of Victoria^[4,6]. At present, most mineral springs in Australia are located within Victoria's central highlands^[5]. Additionally, there are less developed springs, such as those controlled by private land-owners, Indigenous communities, and National Parks including Lorella Springs, Hastings (Tasmania), Butterfly Springs and Mataranka (Northern Territory), where facilities are limited to camping areas and pit toilets^[5].

It has been repeatedly claimed that specific spring water therapies are effective either as a standalone or add-on treatment in treating a range of health conditions, including respiratory conditions^[7,8], osteoarthritis^[9,10], chronic low back pain^[9,11], fibromyalgia^[12], dermatitis^[8], psoriasis^[13], cardiovascular conditions^[8], dyspepsia^[8], obesity^[8], anxiety^[8], and chronic inflammatory pelvic disorders^[8,9]. Despite these assertions, currently spring water therapies do not generally form part of traditional medical treatment in the selected study region.

One of the issues with spring water therapies is the location-specific nature of the mineral water itself. Mineral water differs in terms of the mineral composition and temperature, two proposed mechanisms of the benefits of mineral water. Even the environment in which a treatment takes place may influence benefits. Wohlmann^[14] reported the indications for particular New Zealand springs; for instance, Rotorua's Priest Bath was indicated for those with psoriasis and chronic dry eczema, and the spring water of Te Aroha for chronic gastric catarrh, gout, glycosuria and chronic respiratory catarrh (drinking), and arthritis (bathing). As such, it is important to consider the specific benefits of springs on a local perspective. If spring water sourced from Australia and New Zealand has some of these beneficial effects on human health as demonstrated by systematic review, then medical practitioners and allied health professionals would be able to recommend spring water therapies to their patients in alignment with current evidence-based practice.

The protocol is designed to support a systematic review of studies reporting the health effects of spring water

exposure on human health, using Australia and New Zealand as a case study, in order to answer the following research questions: (1) What are the beneficial human health outcomes associated with exposure to regional spring waters? (2) What are the adverse human health outcomes associated with exposure to regional spring waters?

2 Methods

This protocol has been registered with PROSPERO (June 28, 2015; PROSPERO registration number: CRD42015023713)^[15].

2.1 Eligibility criteria

The search will include studies which investigate or report the health outcomes relating to spring water exposure, using Australian and New Zealand as a case study. The relevance of studies identified through the searches, will be determined using the inclusion criteria reported in Table 1.

2.2 Sourcing and managing studies

Studies will be sourced through database searching, searching specific local journals, and forwards and backwards searching of included studies.

2.2.1 Database searching

Medline (Ovid; 1946–2015), Embase (Ovid; 1947–2015), Allied and Complementary Medicine (AMED; Ovid; 1985–2015), Cochrane Database (1992–2015), Web of Science Core Collection (1900–2015), Cumulative Index to the Nursing and Allied Health Literature (CINAHL; EbscoHost; 1981–2015), PubMed (1966–2015, and selectively from 1809) and Health Source: Nursing/Academic Edition (EbscoHost; 1952–2015) will be searched. These databases have been selected because of their focus on health and medical research. A comprehensive set of search terms were developed by the three authors, after reviewing the search strategies of a number of systematic reviews on similar topics^[7–12,17,18], performing scoping searches, and introductory reading of the topic. Terms were categorised as spring water, exposure, health outcome and Australia/New Zealand terms, with each category combined with the AND Boolean operator (see Appendix 1 for the specific terms, available at <http://www.jcimjournal.com/jim>). No limits will be applied to the searches. If used for other regions, the Australia/New Zealand terms should be changed to reflect that region.

The studies identified in each database search will be exported into EndNote X6. Within EndNote any duplicates will be manually removed, first automatically, and then manually checked. The titles and abstracts of each of the studies in the EndNote library will then be screened for inclusion, according to the above criteria (Table 1). If there is any uncertainty regarding the inclusion of a study the publication will be retained for full text screening. The list of remaining studies will be exported into a Microsoft

Table 1 Inclusion criteria

Item	Criteria
Population	<ul style="list-style-type: none"> Any human population
Exposure	<ul style="list-style-type: none"> Any form of regional (Australian or New Zealand) natural spring water (<i>e.g.</i>, untreated, no added supplements) exposure, including but not limited to bathing, irrigation and drinking Studies which do not specifically state the location of the spring from which the water was taken were included provided the authors had Australian or New Zealand affiliations Studies could include a second intervention, only if there were investigating the added benefit of spring water exposure, or where the comparison group received the same intervention using a different type of water (<i>e.g.</i>, tap water, water from a different spring), so that any health outcome could be attributed to the spring water
Outcomes	<ul style="list-style-type: none"> Any health outcomes, beneficial or adverse, including but not limited to pain, function, quality of life, infections, or poisoning
Others	<ul style="list-style-type: none"> Any study design, except non-systematic reviews[^] Published in English language Published in full text (<i>e.g.</i>, not a conference abstract) Peer-reviewed

This refers to reviews not meeting the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) definition of a systematic review, ‘...a review of a clearly formulated question that uses systematic and explicit methods to identify, select, and critically appraise relevant research, and to collect and analyze data from the studies that are included in the review’^[16].

Excel spreadsheet. The full texts of these remaining studies will then be obtained, and screened for inclusion, with reasons for exclusion recorded.

2.2.2 Google Scholar searching

Google Scholar will be used to search relevant regional journals, in this case three local health/medical journals: *New Zealand Medical Journal*, *Medical Journal of Australia*, and the *Australian and New Zealand Journal of Public Health*. The journal titles will be searched in the publication field, whilst the terms outlined in Appendix 2 (available at <http://www.jcimjournal.com/jim>) will be searched individually.

The studies identified as being potentially relevant, based on title, through the Google Scholar search will be manually entered into a Microsoft Excel spreadsheet. Duplicates within this search will be removed, followed by any duplicates with the database searches. The abstracts of remaining studies will be screened for inclusion using the same criteria as the database search. The full texts of remaining studies will be sourced and then screened with the same criteria, and results will be recorded in the same manner as the database search.

2.2.3 Forwards and backwards searching

The reference lists and citation lists of all included studies from the database and Google Scholar searches will be screened to identify any potentially relevant studies based on the title. Citation lists will be located by searching each of the included studies in Web of Science and Google Scholar. The potentially relevant studies will be manually entered into an Excel spreadsheet, whereby duplicates will be removed first within the results of the forwards and backwards searching, then with the results of the database and Google Scholar searches. Again, the abstracts, then full texts will be screened with the same criteria (Table 1). Within the Excel spreadsheet reasons for exclusion will be recorded, and a list of additional included studies will be developed. If studies are identified through forwards and backwards searching, the process will continue until no further studies are identified.

2.3 Data extraction

Data from each of the included studies will be extracted by two authors, independently, into a purpose-built Excel spreadsheet with the headings reported in Table 2. This

Table 2 Data extraction headings

Item	Criteria
Population	Age; gender; details of the condition (severity, and duration), if applicable
Exposure	Spring location, temperature, setting and composition; type of water application and dosage (duration of exposure, amount of water, and the number and frequency of sessions)
Comparison	As for exposure
Outcomes and outcome measures	Beneficial and adverse health outcomes and the outcome measures used
Others	Year of study (date of submission or publication used if it is not reported); type of research question; study design

process has been pilot-tested with a small sample of international studies relevant to this review topic. After all data extraction has been completed the authors compare their results to ensure accuracy and comprehensiveness.

2.4 Allocating a hierarchy of evidence, and assessment of bias

The included studies will be allocated to the National Health and Medical Research Council (NHMRC) levels of evidence^[19]. Any experimental studies will be assessed for potential bias using the Downs and Black^[20] critical appraisal tool, with cross-sectional, cohort and case-control studies assessed using quality criteria of Shamliyan *et al*^[21]. Due to the inherent biases in case series and case studies, these studies will not undergo critical appraisal. This process will be conducted by two of the authors independently.

2.5 Data reporting and synthesis

A flow-chart based on the PRISMA flow-chart^[16] will be used to report the included and excluded studies.

The extracted data will be reported descriptively in a future publication to identify the types of health outcomes which have been associated with spring water in Australia and New Zealand, any temporal trends, the specific springs and exposure characteristics leading to these health outcomes, and the strength of the evidence, drawing upon the assessment of the risk of bias and hierarchy of evidence classifications. Given the diversity of populations, conditions, exposures and outcome measures it is unlikely that formal quantitative synthesis will be possible. As such, only qualitative, narrative synthesis is proposed.

In the subsequent publication, an overall summary of the strength of the body of evidence identified in this review will be reported using the Grading of Recommendations Assessment, Development and Evaluation (GRADE) criteria^[22]. This will be determined by the authors independently.

3 Discussion

The systematic review proposed incorporates a comprehensive search strategy of eight library databases, in which a thorough list of search terms, including subject headings, will be searched. This will be supplemented by searches of region-specific journals (three major Australian and New Zealand health/medical journals), as well as searching of the reference and citation lists of included studies. It is therefore anticipated that this search will identify all studies which report the health outcomes of human exposure to Australian and New Zealand natural spring water.

In conducting this review, it is expected that recommendations may be developed regarding the safe

and effective use of natural spring waters for a range of health conditions. Such evidence could lead to an increase in the utilisation of spring water, not only amongst tourists, but also as part of a holistic, integrative medical approach, in a similar fashion to that undertaken by QE Health, in Rotorua, New Zealand. The findings of this review will enable medical practitioners and allied health professionals to make evidence-based decisions regarding the use of natural spring water to their patients.

Another outcome of the intended review is to identify gaps in the current evidence base. Such gaps include not only a lack of research evidence on a topic, but also where the current research evidence is insufficient to make a recommendation regarding the safety and effectiveness of natural spring water exposure as a health treatment.

4 Competing interests

The authors declare no competing interests.

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Submission Guide

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