Volume 34 • Issue 1 • **2022**

Australian Journal *of* Herbal and Naturopathic Medicine

A publication of the NHAA

Australian Journal of Herbal and Naturopathic Medicine

nhaa
naturopaths & herbalists
association of australia

The *Australian Journal of Herbal and Naturopathic Medicine* is a quarterly publication of the NHAA. The Journal publishes material on all aspects of Western herbal medicine and is a peer-reviewed journal with an Editorial Board.

ISSN 2209-119X

The *Australian Journal of Herbal & Naturopathic Medicine (AJHNM)* of the Naturopaths and Herbalists Association of Australia (NHAA) is the leading scientific publication of naturopathic and herbal medicine. The NHAA, established in 1920, is the oldest professional association representing naturopaths and herbalists in Australia.

The *AJHNM* publishes key articles to enhance evidence-based herbal and naturopathic medicine in Australia/Western Pacific regions and internationally as an associated journal of the World Naturopathic Federation. The continued growing demand for naturopathic and herbal health services is supported by peer reviewed, relevant evidence that directly informs clinical practice, health policy and higher education. Impact has been achieved through the publication of position papers regarding the eligibility of naturopathic and herbal patients receiving health insurance rebates and through contributions to cementing the professional profiles of herbalism and naturopathic medicine as transparent healthcare professionals.

Aim and scope

The *AJHNM* is a peer-reviewed, academic journal dedicated to publishing all research and articles that relate to naturopathy and herbal medicine practice and policy. The journal publishes all types of article relevant to the profession, from protocols through to meta-analyses and public health issues, including small, specialist commentary and negative studies. Submissions on all aspects of naturopathy and herbal medicine clinical practice and health services are welcome along with research into biological mechanisms of effect of naturopathic approaches and herbal agents, efficacy, safety, patterns of use, cost and implementation.

Editorial policy

Subject material must relate to naturopathy and herbal medicine.

Manuscripts are submitted with the understanding that they have not been published elsewhere or are under consideration for publication elsewhere except in the form of an abstract.

Accepted manuscripts become the property of the *Australian Journal of Herbal & Naturopathic Medicine* and will be copyrighted by the Naturopaths & Herbalists Association of Australia.

Contributions are subject to peer review and editing (in collaboration with authors).

Any author whose name appears on the manuscript assumes responsibility and accountability for the final published manuscript.

By submitting a manuscript to the *Australian Journal of Herbal & Naturopathic Medicine* the author/s agrees to each of the above conditions.

Advertising

For advertising enquiries, please contact the NHAA office on telephone (02) 9797 2244, fax (02) 8765 0091, email nhaa@nhaa.org.au or visit www.nhaa.org.au / Publications and Products / AJHNM

Published four times a year by
Cambridge Media

Publisher Greg Paull
Copy editor Ceridwen Clocherty
Design and layout Gordon McDade

Postal Address
10 Walters Drive Osborne Park, WA 6017
Telephone +61 (0) 8 6154 3911

Follow AJHNM on Facebook
www.facebook.com/pages/Australian-Journal-of-Herbal-and-Naturopathic-Medicine/1416725668550367

Twitter
www.twitter.com/TheNHAA

NHAA postal address:
PO Box 696, Ashfield, NSW 1800

Telephone +61 (0) 2 9797 2244

Fax +61 (0) 2 8765 0091

Email nhaa@nhaa.org.au

Website www.nhaa.org.au

Editor Susan Arentz

Email editorajhnm@nhaa.org.au

CONTENTS

Editorial

Susan Arentz PhD, BHSc(Hons)
Editor, *Australian Journal of Herbal and Naturopathic Medicine*
PO Box 696, Ashfield, NSW 2131, Australia
Email editor.ajhnm@nhaa.org.au

For referencing Arentz S. Editorial. The Australian Journal of Herbal and Naturopathic Medicine 2022;34(1):8-9
DOI https://doi.org/10.33235/ajhnm.34.1.8-9

Welcome 2022, Chinese New Year of the Tiger. This year will see the presentation of the *Health technology assessment (naturopathy)* (HTA) to the Australian government, a significant event that will underpin the definition and role of naturopaths in current healthcare systems. In the President's message, David Casteleijn elaborates on the impact and promise of this important publication. Amongst other things, the HTA will support the government's information about naturopathy, and it will encourage health departments to go beyond the limited acknowledgement of naturopaths we have observed in the pandemic as being included as mandatory vaccination of healthcare workers[1]. Hopefully the new year will pave the way for recognition and opportunity of our growing professions.

Along with the HTA, the *AJHNM* also contributes to improved standing of naturopathy and herbal medicine as expert professions. The HTA describes the *AJHNM* as the third most frequent publication disseminating naturopathic knowledge; with this in mind, it is timely to report on the inroads and direction of *AJHNM*, the scientific journal of the NHAA dedicated to Australian naturopathy and herbal medicine.

Aside from publishing the abstracts of the National Symposium of Herbal and Naturopathic Medicine, over 2020–21 the *AJHNM* continued to receive an increased number of original research submissions. For the first time, we have an ongoing pool of articles that have been accepted following peer review which are ready for publication in subsequent issues. In 2021, three invited articles were published ensuring topical issues that directly affect the naturopathic and herbal medicine clinical practice were covered. Three authors participated in the MACA mentoring program in 2021, developing their manuscripts with one-on-one support. Peer review of all articles was performed by the editorial board, which was expanded to include 15 university-based academics. Their expert recommendations improved the scientific writing of authors, and the transparency of content published in the AJHNM. All of this reflects engagement of naturopaths and herbalists with research and evidence-based practice, and authors openness to transparent feedback as a natural part of the peer review process.

The international scope of the journal has also continued to increase. In 2021 authors from six countries were represented. Although the highest publication rate continued for articles by Australian authors, published articles were by Fijian, Hong Kong, Iranian, Moroccan, and New Zealand based authors. Comparatively, in 2019, no articles submitted by international authors were published.

To support the global impact of naturopathic and herbal medicine, we aim to index the *AJHNM* on the MEDLINE database. In 2021, it was appraised by the National Library of Medicine (NLM) Technical Review Committee (LSTRC) against five criteria (scope and coverage, editorial policies and processes, scientific rigour of article content, production and administration and impact). All domains were assessed as fair to good and the reviewers recommended that we reapply following specific improvements. Many of the suggested improvements have been addressed, including increased publication of original research, less solicited commentary, and a more professional diversity of the editorial board. We continue to work towards increased visibility of consent, and authors' declarations of interests. Although work remains, the improvements thus far have contributed to raising the *AJHNM*'s impact ranking. The pharmacology (nursing) category of the Scopus database (Elsevier B.V.) reports the *AJHNM* as being ranked in the Q2 category, up by two quartiles from Q4 in 2019. Thank you for interest in the *AJHNM* and for your work and contributions; it really does enrich the journal and contribute to the intellectual rigour of naturopathy and herbal medicine.

This issue includes three articles as well as a celebration of 60 years of the Southern School of Natural Therapies.

With over 1000 students currently enrolled, and with such a long history, the institution is the backbone of the profession. Not only does it continue to flourish, but the founders, the Jacka family, continue to fund the lion's share of naturopathic research and development. It is a pleasure to publish the 60-year celebration. I hope you enjoy.

This issue includes an overview of evidence for medicinal mushrooms as adjunct therapy in women with breast cancer. The article compiles the pre-clinical and clinical research and guides the direction of further research. *Ganoderma lucidum* was used to boost immunity and reduce cancer-related fatigue.

Also included is a clinical trial comparing inhalation of essential oils (rose) against Metoclopramide for depression and anxiety in pregnant women with nausea and vomiting. There is a gap in the clinical management of these women as unwanted side effects of Metoclopramide, including sleepiness, fatigue and restlessness, are common. Pregnant women prefer natural types of treatment approaches.

Also included a laboratory-based investigation into the efficacy of essential oils on growth and biofilm formation of *Candida albicans*. Essential oils included *Matricaria chamomilla, Urtica dioca, Trigonella foenum-graecum* and the honeybee product propolis oil as well as other traditional Jordanian herbal medicines including *Nigela sativa.* The study presents a strong case for subsequent clinical investigation into using essential oils as treatment for complicated Candida infections in humans.

Dr Wendy McLean has again put together the MedJourn and MedHerb sections summarising the recently published evidence for herbal medicines and naturopathic treatments. Please find the continuing professional development questions at the end of this issue.

Reference

1. NSW Health. Vaccination order extended to primary care and private sector health service providers. Available from: www.health.nsw.gov.au/news/Pages/20211216_01.aspx2022.

As we go to publication, sad news of Judy Jacka passing away has been posted by the Jacka Foundation. Judy is the founder of the Jacka Foundation and a long standing, committed stalwart and advocate of education and research in natural therapies. We take the opportunity to acknowledge Judy's incredible foresight and her generous philanthropic activities and organisation that has provided many opportunities and made an enormous contribution to the advancement of naturopathy and herbal medicine. https://www.jackafoundation.org.au/images/Announcement_of_Judys_Passing.pdf

Australian Journal of Herbal and Naturopathic Medicine **2022** 34(1)

President's message

David Casteleijn BHSc(Nat), MHSc (Herbal Med), RN
President NHAA
PO Box 696, Ashfield, NSW 1800, Australia
Email president@nhaa.org.au

For referencing Casteleijn D. President's message. The Australian Journal of Herbal and Naturopathic Medicine 2022;34(1):10

DOI https://doi.org/10.33235/ajhnm.34.1.10

What a wonderful time to be a naturopath or herbalist. In the last week of May we hope you will join us for the Herbal Medicine Summit in Sydney. We are presenting a wonderful range of Australian and international speakers. Excitingly, we have a program which spans all the way from connecting with our herbal medicine roots through to looking to the future with supporting cutting edge research.

But before the Herbal Medicine Summit we launch the *Health technology assessment (naturopathy)* (HTA) at the end of this month. On 25 March the NHAA will join other organisations from around the world in a global launch of this incredible document. In Australia the launch will be at Parliament House in Sydney, with our aim to bring the wonderful world of naturopathy to the attention of government and policymakers across the country.

The World Naturopathic Federation (WNF) first considered creating an HTA for naturopathy in 2017 as its development was crucial for the future prosperity of the international naturopathic profession. The Naturopaths and Herbalists Association of Australia (NHAA) considered this project of such significance they chose to fund the research component and are delighted to offer the HTA as a gift to the naturopathic profession, not only in Australia but across the world.

The protocol and methods for the HTA follow the WHO HTA guidelines. The scope of the HTA was informed by research conducted by the international naturopathic community over the last 30 years encompassing over 2000 peer-reviewed scientific articles of which more than 300 clinical studies involving over 100 different health populations are outlined in the HTA. The studies included complex interventions based on holistic, patient-centred, multi-modality treatment – a hallmark of naturopathic care.

The HTA is a wonderful opportunity for the naturopathic profession to not only highlight the great work we are doing but, more importantly, the great work naturopathy could be doing if it were more integrated into the overall healthcare system – highlighting the potential as well as the actual practice of naturopathy. Because it is a government recognised format, it has its own credibility, with the HTA consolidating the research and knowledge we have on the profession. The HTA is an opportunity for the naturopathic profession to clarify who we are and what we do.

The outcomes of the HTA align with the *Declaration of Astana* report[1] and demonstrate that naturopathic medicine is a safe and effective intervention that has utility across different geographic regions, clinical settings and conditions, and that naturopathic practitioners are trusted and consulted by the global public. Studies demonstrate the clinical effectiveness and efficacy of naturopathic interventions in a wide variety of conditions combined with cost-effectiveness studies, suggesting integration of naturopathic care can generate cost savings at individual clinic and health systems levels.

Definitive conclusions on the effectiveness of naturopathy/naturopathic medicine are hampered by the lack of integration of naturopathy/naturopathic medicine into broader healthcare, research or academic initiatives. Nevertheless, despite such barriers, particularly in areas of global health priority such as non-communicable diseases, naturopaths have been actively engaged in both the conduct and translation and implementation of research which provides a solid foundation for future integration into future clinical and research endeavours.

By now I am sure you will be very keen to read more of the HTA for yourself; my suggestion is to head over to the dedicated page on the NHAA website[2]. Perhaps start with the Synopsis and the Executive summary and, if you are super keen, you can find links to the full document here too.

References

1. WHO. Declaration of Astana. Available from: https://www.who.int/docs/default-source/primary-health/declaration/gcphc-declaration.pdf
2. Naturopaths & Herbalists Association of Australia (NHAA). HTA – Health Technology Assessment. Available from: https://nhaa.org.au/news/hta-health-technology-assessment/

Australian Journal of Herbal and Naturopathic Medicine **2022** 34(1)

Milestone in Australian naturopathic education: Southern School of Natural Therapies celebrates 60 years

Catherine Smith (Prof Hons Complementary Medicine)
Torrens University, NSW, Australia

Dr Greg Connolly PhD
Torrens University, NSW, Australia

Natalie Cook Masters (Public Health)
Torrens University, NSW, Australia

Contact information
Catherine Smith, Program Director, Naturopathy & Western Herbal Medicine, Torrens University, Pyrmont, NSW
Email clsmith@torrens.edu.au

Keywords naturopathy, western herbal medicine, education, Southern School of Natural Therapies

For referencing Smith C et al. Milestone in Australian naturopathic education: Southern School of Natural Therapies celebrates 60 years. The Australian Journal of Herbal and Naturopathic Medicine 2022;34(1):24-26

DOI https://doi.org/10.33235/ajhnm.34.1.24-26

Submitted December 2021*, Accepted* February 2022

Abstract

Southern School of Natural Therapies (SSNT) is Australia's longest standing school of natural therapies. Founded in 1961 by Alf Jacka, the school has been in continuous operation since its inception 60 years ago. SSNT was founded with a vision for elevating naturopathic educational standards and it has been a leader in this field over decades. The purpose of this article is to reflect on the contribution of SSNT to naturopathic education and to look towards to the bright future ahead.

The College commenced operations on 6 February 1961[1(p80)] under the name of Victorian Branch College of the National Association of Naturopaths, Osteopaths and Chiropractors (NANOC)[2(p13)]. The inaugural Dean was pioneer naturopath Alf Jacka who set up the school that was initially housed at his East Melbourne clinic. Alf was encouraged to set up the College by friend, colleague and Australian naturopathic icon, Maurice Blackmore (1906–1977). Maurice then became a College mentor and designed the College final year exit exam that consisted of 1,000 questions to be answered by all students before graduation, an impressively high bar to set in those early days. In the early days, Alf taught all the classes following a full day of clinical practice; classes were 2 hours' duration, 4 nights per week. That first library was housed in a small steel cabinet, whilst the Alf Jacka library at SSNT today is considered the largest of its type in the southern hemisphere! The curriculum consisted of 50 booklets of approximately 20 pages covering anatomy and physiology, chemistry, symptomatology, mineral therapy, herbal medicine and iris diagnosis[2(p14)]. Clinical prescribing often involved a liquid herb mix, compounded herbal tablets, homeopathy and celloids.

Judy Jacka, originally a trained nurse, studied at the College from 1969–1971 and went on to become synonymous with the college. She took on teaching roles following her studies and subsequently became principal of the school from 1974–1985 and Chairperson until 1996[3]. Under her influence, the College quickly grew and relocated to new premises in Kew[1(p30)]. Enrolments increased from around 10 students in 1961 to 53 students enrolled in 1973 (to closer to 1000 in 2019). The course hours increased to 1400 hours to accommodate a more formal and scientifically focused curriculum. Blending traditional knowledge with science resulted in an expanded curriculum including naturopathic philosophy, nutritional and botanic clinical medicine across the

lifespan, pathology and diagnostics, biochemistry, homoeopathy, chiropractic, osteopathy, jurisprudence and sociology. In 1973, course fees were $2,000 per year with lecturers paid approximately $12.50 per hour[2(pp36–7)].

In 1978 the College relocated to a historic building in Victoria Street, Fitzroy[2(p58)]. Over time, the campus improved with bespoke classrooms, lecturer and student spaces, customised clinic, expansive library and a herb garden. The course was now full-time and students attended for 30 lecturing hours per week. In 1981 the College name changed to Southern School of Natural Therapies (SSNT)[1(p30)]. In the same year, the College was approved as a not-for-profit company. This altruistic business model set the tone for improving the credibility of natural therapies' educational institutions in the eyes of the government and public[2(p79)]. The curriculum was used by the Australian Natural Therapists Association (ANTA) to gain health fund rebates for naturopathic practitioners in the 1980s.

During this time, SSNT raised educational standards significantly. Improvements were accelerated when, in 1977, the Victorian Government released the Webb Report where it was noted that naturopathy lacked sufficient scientific and biomedical subjects in its curriculum[4].

Teaching staff over the years. L–R: Ondine Spitzer, Sue Buckle, Judy Singer, Assunta Hunter, Jan Batty, Sue Evans, Greg Connolly

Teaching staff over the years. L–R: Andree Semmens, Jenny Adams, Gabe Covino, Liza Oates, Nicole Quaife

Judy Jacka immediately established committees to design a 4-year full-time industry-recognised Diploma of Applied Science Naturopathy qualification. This course incorporated medical sciences with naturopathic subjects and began in 1982.

In 1997, SSNT was the first school in Victoria and the first Australian private education provider to gain unconditional degree accreditation for the Bachelor of Health Science (Naturopathy)[2(p167)]. These milestones were hard won, with many government challenges to SSNT's integrity, level of training and naturopathy's value and relevance to the healthcare system. Judy Jacka was instrumental in coordinating a number of submissions on behalf of SSNT and the naturopathic profession to oppose various legislations that threatened the future of naturopathy and natural therapies. Through persistence, belief and passion, our early SSNT pioneers successfully elevated the educational standards and reputation of the naturopathic profession.

SSNT's influence rapidly expanded nationally and internationally in the 2000s. SSNT's educational enterprise was sold to Think Education Group (THINK) in 2010 and in 2013 Laureate International Universities acquired THINK. During this time, SSNT became part of an Australian wide network of colleges. The Australasian College of Natural Therapies (ACNT) launched the SSNT curriculum for the Bachelor of Health Science (Naturopathy) in Sydney and Brisbane during 2014.

SSNT 60th birthday celebrations

Natalie Cook, Bryce Ives, Kath Curry

Being part of a world-wide network of Laureate higher education institutions provided the academics and students the opportunity to collaborate with naturopathic schools from around the world. Joint case discussions in which students and educators shared contextualised herbal and naturopathic knowledge and treatment approaches were conducted with the *Universidade Anhembi Morumbi,* Brazil in 2018. In 2019, a delegation of Japanese Medical Herb Association members visited SSNT to learn about western herbal medicine. SSNT also joined the World Naturopathic Federation (WNF) as an educational member in 2019 and hosted their international General Assembly. SSNT's global influence continues to expand with international alumni in areas such as Asia, Latin America and Europe.

In line with Judy and Alf Jacka's vision for pursuing the highest level of educational standards, the Bachelor of Health Science (Naturopathy) course was launched at Torrens University Australia (TUA) in 2019. This bought naturopathic higher education back into the university sector, again raising the profile and reputation of naturopathic education in the eyes of the government and regulators. The course underwent a major update in 2020 to align the course structure and to incorporate an innovative work integrated learning program.

In 2020 TUA changed ownership and is now proudly part of the US-based Strategic Education Inc. family. Recent challenges of the COVID-19 pandemic have seen the legacy of innovation continue from those SSNT foundations as the program was required to rapidly pivot to accommodate the changed learning environment. This included, as necessary, flexible online learning, workshop intensives for practical skills and an innovative Telehealth clinical practicum. Future developments involve promoting post-graduate research opportunities aligned to the university's Be Good & Be Well strategy.

Throughout these changes, the constant foundation of the university has been the people. True to SSNT tradition, students became teachers and return to share their knowledge. SSNT is proud to have nurtured many educators over the years, some of whom have more than 35 years' experience in teaching and clinical practice. This shared wisdom allows the traditions of naturopathy to shine alongside contemporary science, making graduates stronger, more confident, and able to work in diverse practices, including integrative and collaborative models of care.

SSNT's distinctive friendly, energetic and passionate community of staff and students has shaped the TUA culture. The beloved Fitzroy campus has plenty of creative learning spaces, encouraging collaboration and socialisation. This positive environment has contributed to SSNT not only being a place of excellence in naturopathic and herbal medicine training, but also a place where mentoring and friendships thrive well beyond graduation. A hallmark of being an SSNT alumni is not just achieving the highest standard of formal education, but also being supported by a strong network of colleagues, many of whom have gone on to become leaders in the health sector in Australia and around the world.

To celebrate 60 years and to honour the vast contribution of SSNT founder Alf Jacka, a full annualised scholarship has been established for BHSc (Naturopathy) students. SSNT today may have exceeded even the expectations of Alf and Judy Jacka. Their legacy lives on in the people and values of TUA that is built on the shoulders of giants like SSNT and will continue to shape the next generation of health professionals.

Conflict of interest

Catherine Smith, Greg Connolly and Natalie Cook are employed by Torrens University Australia, the owner of Southern School of Natural Therapies.

Funding

The authors received no funding for this study.

References

1. Baer HA. Complementary medicine in Australia and New Zealand. Maleny, Australia: Verdant House; 2009.
2. Jacka J. Natural therapies: the politics and the passion. Ringwood, Australia: Ringwood Natural Therapies; 1998.
3. The Jacka Foundation of Natural Therapies. Board profiles: Judy Jacka. Available from: https://www.jackafoundation.org.au/about/board-profiles
4. Evans S. The story of naturopathic education in Australia. Complementary Therapies in Medicine 2002;8:243–240.

Novel essential oils target the virulence factors of *Candida albicans*

Homa Darmani*, Ahmad T Elwan, Abdulrahman M Abu Shaban

Department of Biotechnology and Genetic Engineering, Faculty of Science and Arts, Jordan University of Science and Technology, Jordan

Contact information

Professor Homa Darmani, Department of Biotechnology and Genetic Engineering, Faculty of Science and Arts, Jordan University of Science & Technology, P.O. Box 3030, Irbid 22110, Jordan
Email darmani@just.edu.jo

Keywords antifungal agents, morphogenesis, biofilms, mitochondrial activity

For referencing Darmani H et al. Novel essential oils target the virulence factors of *Candida albicans*. The Australian Journal of Herbal and Naturopathic Medicine 2022;34(1):28-33

DOI https://doi.org/10.33235/ajhnm.34.1.28-33

Submitted 16 January 2022, Accepted 15 February 2022

Abstract

Candida albicans (C. albicans) biofilm-related diseases remain a major challenge for clinicians due to the formidable and increasing resistance to commonly used antifungal drugs. This has led to the search for new phytotherapeutic modalities like the use of medicinal plants and their extracted essential oils. We examined the effects of eight plant essential oils on the growth (disc diffusion assay), mitochondrial activity (MTT assay), germ tube and biofilm formation (crystal violet assay) of *C. albicans.* We found that *Urtica dioica (U. dioica)* essential oil significantly reduced the growth of *C. albicans,* and *Saussurea costus (S. costus), Boswellia serrata (B. serrata)* and *Commiphora myrrha (C. myrrha)* significantly reduced mitochondrial dehydrogenase activity ($p<0.0001$). We report, for the first time, that *U. dioica*, *S. costus* and *Matricaria chamomilla* essential oils also caused potent inhibition of germ tube formation, and *C. myrrha* and *S. costus* essential oils exerted strong inhibition of biofilm formation ($p<0.0001$). Since morphogenesis and biofilm formation are vital for *C. albicans* to penetrate and invade tissues and to persist as antimicrobial tolerant cells in the biofilms, these essential oils may present an additive, and less toxic, approach to standard antifungal drugs.

Introduction

The opportunistic dimorphic pathogen *Candida albicans* (*C. albicans*) is part of the normal flora of the skin and mucosal membranes of the oral, genital and gastrointestinal tracts of the majority of healthy people[1,2]. The transition from symbiotic to parasitic mode during times of dysbiosis and immune suppression results in infections that range from superficial to debilitating and life-threatening invasive disease with substantial morbidity and mortality of affected patients[1,3]. The pleomorphic nature of *C. albicans* is one of the key determinants of its pathogenesis and this ability to shift reversibly from yeast to pseudohyphal and true hyphal forms is important in both the ability to colonise and parasitise host tissues[2]. The hyphal form is associated with adhesion and invasion of host tissues and the yeast form with bloodstream dissemination[4].

The formation of Candida biofilms on abiotic and biotic surfaces, indwelling medical devices and host cells, respectively, is also of clinical concern due to the increased resistance to antimicrobial agents. Estimates suggest that 65–80% of all human microbial infections involve pathogenic biofilms[5,6]. Biofilms represent elaborate surface-linked yeast communities nested within extracellular polymeric substances and account for the formidable resistance to commonly used antifungal drugs[7,8].

Conventional antifungal therapy has limitations with respect to efficacy, toxicity, drug interaction and emergence of resistant microorganisms, even in planktonic form[9]. Although the benefits of medicinal plants and their extracted essential oils has been known since antiquity, research into the antimicrobial properties has increased only during the recent years. Phytotherapeutic approaches may present an alternative, more effective and less toxic strategy to standard antifungal drugs[10,11].

In the current study, the anticandidal effects of eight different essential oils were investigated namely: *Nigella sativa* (*N. sativa*); *Saussurea costus* (*S. costus*); *Commiphora myrrha* (*C. myrrha*); *Matricaria chamomilla* (*M. chamomilla*); *Trigonella foenum-graecum* (*T. foenum-graecum*); *Urtica dioica* (*U. dioica*); *Boswellia serrata* (*B. serrata*); and Propolis.

Materials and methods

Essential oils

The essential oils tested in this study were: black seed (*N. sativa*); costus root (*S. costus)*; myrrh (*C. myrrha*); chamomile (*M. chamomilla*); fenugreek (*T. foenum-graecum*); nettle (*U. dioica*); ilibanum (*B. serrata)* and a commercially prepared Propolis oil. All the essential oils were purchased from the local market in Amman, Jordan.

Disc diffusion test

The effects of the different essential oils on the growth of *C. albicans* was tested using the agar disk diffusion Kirby-Bauer sensitivity test[12]. Sterile 5mm diameter filter paper disks were placed on Sabouraud dextrose agar (SDA) (Oxoid Ltd, Cheshire, England) previously seeded with 10µl of SAB broth culture of *C. albicans* (ATTCC 10231) ($1x10^6$ cells/mL). Following this, 20µl of each essential oil (diluted 1:1 in dimethyl sulfoxide (DMSO)) (Sigma-Aldrich Inc., Minnesota, USA), or for the controls, DMSO and amphotericin B (Sigma-Aldrich Inc.), was dispensed onto each disk. For the essential oils, the discs contained a final concentration of 10mg and 5µg for the amphotericin B. The plates were then incubated at 37˚C for 24 hours and any zones of inhibition of growth were measured.

MTT assay

The essential oils were dissolved in DMSO at a ratio of 1:1 and then diluted in SDB to a final concentration of 0.02% which is equivalent to 200µg/mL. Similarly, the DMSO and amphotericin B controls were prepared in SDB. Broth cultures of *C. albicans* (50µL of $1x10^6$ cells/mL) were exposed to 50µL of the essential oils or DMSO or amphotericin B in 96-well plates and incubated at 37˚C for 24 hours in a rotary incubator at 75rpm. An aliquot of 10µL of MTT (Sigma-Aldrich Inc.) (3mg/mL in phosphate buffered saline) was added and the cells incubated at 37˚C for 3 hours and then the resulting formazan crystals were dissolved with an equivalent volume of DMSO (110µL) and gently mixed in a rotary incubator for 30 minutes and the absorbance measured at 580nm using a microplate reader (Multiskan Plus EFIAB, Titrek).

Germ tube formation

The effects of the essential oils on germ tube formation was investigated according to the technique described by Mackenzie[13] with minor modifications. An aliquot of 20µL of *C. albicans* ($1x10^6$ cells/mL) was exposed to the different essential oils (1.8mL) (4 replicates) in 24 well plates and incubated at 37ºC for 24 hours in a rotary incubator at 75rpm. Controls consisted of *C. albicans* exposed to DMSO and amphotericin B. An aliquot of 200µL of horse serum (10%) (Sigma-Aldrich Inc.) was added and following incubation at 37ºC for 3 hours in an orbital shaking incubator, slides were prepared for each sample and 100 randomly selected cells were enumerated for germ tube formation and considered germinated if they had a germ tube at least twice the length of the cell. All the experiments were repeated three times.

Biofilm formation

The effects of the essential oils on biofilm formation was investigated as described previously with minor modifications[14]. *C. albicans* ($1x10^5$ cells/mL) was exposed to different essential oils in 24 well tissue culture treated plates and incubated at 37˚C for 72 hours in a rotary incubator at 75rpm to allow biofilm formation. The medium was then discarded and the wells were washed three times, after which the biofilms were stained with 1mL crystal violet solution (0.4%) for 15 minutes. The biofilms were rinsed carefully and, once dry, the stained biofilms were solubilised with acetic acid (10%) and 100µL aliquots transferred to a 96-well plate and the absorbance measured at a wavelength of 600nm in a microplate reader.

Statistical analysis

Data are expressed as means ± standard deviation. Differences between the groups were analysed using one-way Analysis of Variance (ANOVA) and Tukey's Pairwise Comparisons (confidence intervals (CI) of 95%). A p value less than 0.05 was considered to be significant.

Results

Since there is increasing interest in the search for innovative plant-derived antifungal agents as alternative treatments for fungal infections, the current study examined the effects of different essential oils on the growth, mitochondrial activity, germ tube and biofilm formation of *C. albicans*. Figure 1 shows the results of the disc diffusion assay. It can be seen that, of the eight essential oils tested using the disc diffusion assay, only *U. dioica* exerted inhibition of growth of *C. albicans*. Although the size of the inhibition zone was greater than that induced by the antimycotic agent amphotericin B, the difference was not statistically significant.

Figure 2 shows that nearly all essential oils caused decreased mitochondrial activity relative to the controls (100%), with the exception of *N. sativa* essential oil which caused increased mitochondrial activity. One-way ANOVA revealed significant effects on mitochondrial activity (relative to control DMSO) of *C. albicans* ($p<0.0001$). Tukey's pairwise comparisons showed significant decreases in mitochondrial activity of *C. albicans* exposed to essential oils from *S. costus*, *B. serrata*, *C. myrrha*, as well as amphotericin B, relative to the DMSO controls. The greatest reduction in mitochondrial activity was observed with amphotericin B, followed by *C. myrrha*, *B. serrata* and *S. costus.* On

the other hand, exposure of *C. albicans* to the essential oil from *N. sativa* caused a significant increase in mitochondrial activity.

Figure 3 shows that essential oils from *C. myrrha*, Propolis and *N. sativa* did not affect germ tube formation when compared to the controls. However, exposure to the other essential oils reduced germ tube formation of *C. albicans*. One-way ANOVA showed statistically significant effects between the average number of germ tubes formed following the different treatments ($p<0.0001$). Tukey's pairwise comparison revealed significant reductions in germ tube formation of *C. albicans* exposed to essential oils from *T. foenum-graecum*, *S. costus*, *B. serrata, U. dioica* and *M. chamomilla* in comparsion to *C. albicans* exposed to the DMSO control. The greatest reduction in germ tube formation was observed with amphotericin B, followed by *U. dioica, M. chamomilla*, *B. serrata* and *T. foenum-graecum* (Figure 3).

Figure 4 shows that essential oils from *N. sativa*, *S. costus, C. myrrha, U. dioica, B. serrata* and Propolis caused a reduction in biofilm formation when compared to the control. On the other hand, exposure to the essential oils from *M. chamomilla* and *T. foenum-graecum* increased biofilm formation. One-way ANOVA revealed highly significant differences between the biofilm formation of *C. albicans* in the different treatment groups ($p<0.0001$). Tukey's pairwise comparison (Figure 4) revealed significant reductions in biofilm formation of *C. albicans* exposed to essential oils from *N. sativa*, *S. costus, C. myrrha, B. serrata* and Propolis in comparison to *C. albicans* exposed to the DMSO control. The greatest reduction in *C. albicans* biofilm formation was observed with amphotericin B, followed by essential oils from *B. serrata, S. costus, C. myrrha,* Propolis, *N. sativa*, and *U. dioica* (Figure 4).

Discussion

Since there is increasing interest in the search for innovative plant-derived antifungal agents as alternative treatments for fungal infections, the current study examined the effects of different essential oils on the growth, mitochondrial activity, germ tube and biofilm formation of *C. albicans*. We found that the essential oil

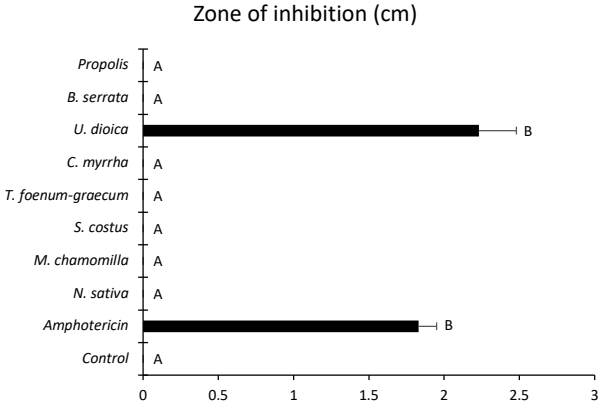

Figure 1. The effects of essential oils and amphotericin B on the growth of *C. albicans**

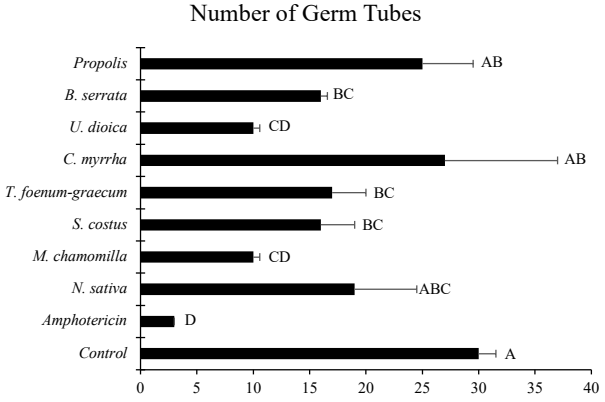

Figure 3. The effects of essential oils and amphotericin B on the germ tube formation of *C. albicans**

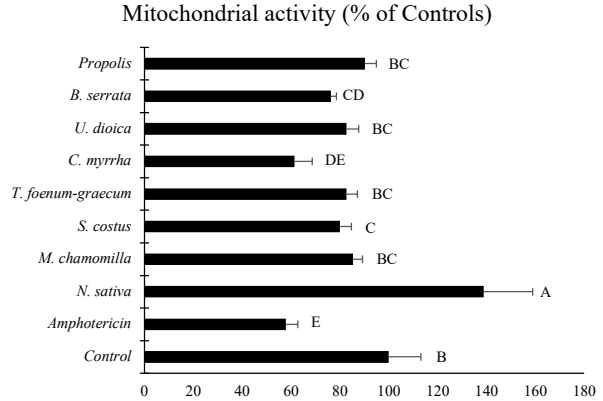

Figure 2. The effects of essential oils and amphotericin B on the mitochondrial activity of *C. albicans**

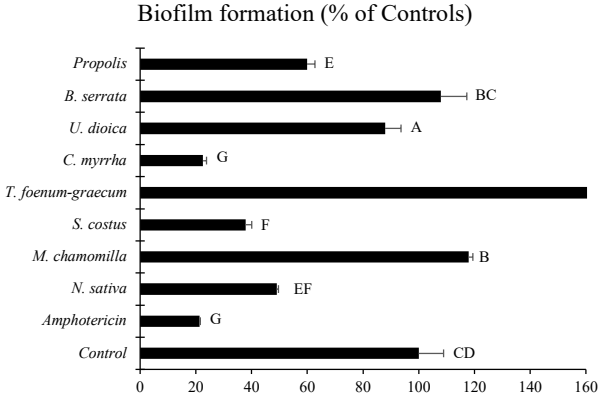

Figure 4. Effects on biofilm production of *C. albicans**

*Data are presented as means + standard deviation. Bars that do not share a letter are significantly different (Tukey's pairwise comparisons)

from *N. sativa* did not affect the growth of *C. albicans* but it caused a significant reduction (45%) in biofilm formation compared to the control (100%). These results are in agreement with a previous study that found that *N. sativa* extract was not fungicidal in a mouse model of candidiasis, but the extract reduced the number of adherent colonies of the organism in the kidneys, liver and spleen from a 5-fold to 11-fold decrease[15]. Furthermore, Thymoquinone, the major ingredient of *N. sativa* essential oil, when incorporated into PMMA denture base, reduced the adhesion of *C. albicans* to denture materials thus reducing biofilm formation[16]. The thymoquinone in *N. sativa* essential oil may thus be the reason for inhibition of *Candida* adhesion and biofilm formation.

The essential oil from *M. chamomilla* exerted no effects on the growth of *C. albicans*, in agreement with a previous study[17]. However, exposure of *C. albicans* to *M. chamomilla* essential oil caused a 65% reduction in germ tube formation and this agrees with another report which showed that *M. chamomilla* oil strongly inhibited germ tube formation but caused a slight inhibition in the growth of this yeast[18]. The major components of *M. chamomilla* essential oil are reported to be α-bisabolol oxide (38%), bisabolol (21%) and farnesene (19%)[19]. Antimicrobial activities of the essential oil have been linked with the active components α-bisabolol oxide and bisabolol[20]. Furthermore, α-bisabolol has been reported to inhibit ergosterol synthesis in *C. albicans*[21]. The effect of *M. chamomilla* on germ tube formation, in the current study, may be due to the third major component farnesene which is reported to inhibit the morphological transition of *C. albicans*[22].

The essential oil from *S. costus* had no effects on the growth of *C. albicans*, in agreement with a recent study that reported minimal effects on the growth of *C. albicans*, although significant antifungal activity was observed against *Aspergillus niger*[23]. However, in the current study we report, for the first time according to our knowledge, that *S. costus* essential oil caused significant reductions in mitochondrial activity, germ tube formation and biofilm formation of *C. albicans*. The sesquiterpene dehydrocostus lactone is the second major component of *S. costus* essential oil and has been reported to have various biological activities including antifungal effects[24,25]. However, there have been no studies on the effect of dehydrocostus lactone on the virulence factors of *C. albicans* and this needs further investigation.

T. foenum-graecum essential oil did not have any effects on the growth *C. albicans*, in contrast to another study which showed that ethanolic extracts of *T. foenum-graecum* seeds exerted antifungal effects against *C. albicans*[26]. However, we found that exposure of *C. albicans* to *T. foenum-graecum* essential oil led to nearly 50% reduction in germ tube formation. The effects on germ tube formation have not been previously reported, according to our knowledge. The main component of *T. foenum-graecum* essential oil is reported to be (2E)-Hexenal (26.61%)[27]. However, Thymol (4.79%) is also present and may be the contributing factor for the decreased germ tube formation since it is reported to inhibit the filamentous growth of *C. albicans*[28].

C. myrrha essential oil caused significant reductions in mitochondrial dehydrogenase activity of *C. albicans* leading to impaired energy production capacity. Furthermore, a significant reduction in biofilm formation (62%) was also observed. These effects of *C. myrrha* essential oil on *C. albicans* have not been previously reported, according to our knowledge. The most prominent compounds of *C. myrrha* essential oil are reported to be furanoeudesma-1, 3-diene (17.65%), followed by curzerene (12.97%), β-elemene (12.70%), germacrene B, D and A, 12.15%, 9.13% and 5.87%, respectively[29]. Curzerene and β-elemene have antifungal activity' however, the effects of these components of *C. myrrha* essential oil on *C. albicans* have not been previously reported, according our knowledge, and needs further investigation[30,31].

U. dioica essential oil inhibited the growth of *C. albicans*, and the inhibition of growth was greater than amphotericin B. These results agree with another study that reported that an alcoholic extract of *U. dioica* leaf has anti-candidal activity[32]. The monoterpene carvacrol is the major component of different essential oils including *U. dioica* (38.2%) and has been reported to exert remarkable antifungal and antibacterial effects[33,34]. Carvacrol is reported to affect Ca^{2+} and H+ homeostasis, leading to loss of ions and interference in the TOR signaling pathway, resulting in loss of viability of *Saccharomyces cerevisiae*[35]. In *C. albicans*, Carvacrol alters the integrity of the ER, culminating in ER stress and the triggering of the unfolded protein response (UPR) to buffer this stress[33]. Since several UPR target genes are induced during the yeast-to-hyphae transition, exposure of *C. albicans* to carvacrol in *U. dioica* essential oil may result in up- and down-regulation of gene transcription which maybe the reason for impairment of the ability of *C. albicans* to switch to the hyphal form[36]. In the current study we observed a 67% decrease in germ tube formation of cells exposed to *U. dioica* essential oil, relative to the DMSO controls.

B. serrata essential oil caused significant reductions in mitochondrial dehydrogenase activity of *C. albicans* and this effect is probably due to α-pinene, the major component (74–80%)[37] which has also been reported to cause decreased mitochondrial activity in *Saccharomyces cerevisiae*[38]. Since germ tube formation is an energy-dependent process, it is not surprising that exposure of *C. albicans* to *B. serrata* essential oil also caused decreased filamentation.

Propolis essential oil caused decreased biofilm formation (40% reduction), in agreement with a previous study which reported that propolis decreased *C. albicans* biofilm formation *in vitro* and propolis based gels and creams were partially able to control vulvovaginal

candidiasis in a mouse model[39]. The effects on biofilm formation may be due to the presence of α-pinene as the major constituent of Propolis essential oil (18.3%)[40] which has been reported to actively inhibit *C. albicans* biofilm formation[41].

Conclusion

It has been well established that the transition from yeast to hyphal morphology and biofilm formation are important for the ability of *C. albicans* to penetrate and invade tissues and for the persistence of antimicrobial tolerant cells in the biofilms[7,39,40,42,43]. Targeting the virulence factors of *C. albicans* by essential oils that strongly inhibit phenotypic switching and biofilm formation would be an added armamentarium in the fight against cadidiasis. The full potentials of these essential oils as novel anti-fungal agents, possible synergistic effects with amphotericin B, the spectrum of activity against different strains of Candida, and mechanisms of action of the essential oils and their major components should be further exploited.

Conflict of interest

The authors declare no conflicts of interest.

Funding

This work was supported by the Deanship of Research at Jordan University of Science and Technology (Grant Numbers: 20150257; 20160071; 20160080).

References

1. Pérez JC, Kumamoto CA, Johnson AD. *Candida albicans* commensalism and pathogenicity are intertwined traits directed by a tightly knit transcriptional regulatory circuit. PLoS Biology 2013;11:e1001510.
2. Hall RA, Noverr MC. Fungal interactions with the human host: exploring the spectrum of symbiosis. Current Opinions in Microbiology 2017;40:58–64.
3. Huseyin CE, O'Toole PW, Cotter PD, et al. Forgotten fungi: the gut mycobiome in human health and disease. FEMS Microbiology Review 2017;41:479–511.
4. Wilson D, Naglik JR, Hube B. The missing link between *Candida albicans* hyphal morphogenesis and host cell damage. PLoS Pathogens 2016;12:e1005867.
5. Lynch AS, Robertson GT. Bacterial and fungal biofilm infections. Annual Review of Medicine 2008;59:415–428.
6. Ramage G, Mowat E, Jones B, et al. Our current understanding of fungal biofilms. Critical Reviews in Microbiology 2009;35:340–355.
7. LaFleur MD, Kumamoto CA, Lewis K. *Candida albicans* biofilms produce antifungal-tolerant persister cells. Antimicrobial Agents and Chemotherapy 2006;50:3839–3846.
8. Wu S, Wang Y, Liu N, et al. Tackling fungal resistance by biofilm inhibitors. Journal of Medicinal Chemistry 2017;60:2193–2211.
9. Ruhnke M. Antifungal stewardship in invasive Candida infections. Clinical Microbiology and Infection 2014;20:11–18.
10. Alves CT, Ferreira IC, Barros L, et al. Antifungal activity of phenolic compounds identified in flowers from north eastern Portugal against Candida species. Future Microbiology 2014;9:139–146.
11. Teodoro GR, Ellepola K, Seneviratne CJ, et al. Potential use of phenolic acids as anti-Candida agents: a review. Frontiers in Microbiology 2015;6:1–11.
12. Hudzicki J. Kirby-Bauer disk diffusion susceptibility test protocol. American Society for Microbiology, Washington DC, 2012;1–13. Available from: https://www.asm.org/Protocols/Kirby-Bauer-Disk-Diffusion-Susceptibility-Test-Pro
13. Mackenzie DWR. Serum tube identification of *Candida albicans*. Journal of Clinical Pathology 1962;15:563–565.
14. Taweechaisupapong S, Ngaonee P, Patsuk P, et al. Antibiofilm activity and post antifungal effect of lemongrass oil on clinical Candida dubliniensis isolate. South African Journal of Botany 2012;78:37–43.
15. Khan MA, Ashfaq MK, Zuberi HS, et al. The in vivo antifungal activity of the aqueous extract from Nigella sativa seeds. Phytotherapy Research 2003;17:183–186.
16. Albarrag AM, Alothman OY, Elsharawy MA, et al. Effect of *Nigella sativa* extracts on candida species adhesion to acrylic denture base material and on nanomechanical properties. Jnl Sc Adv Materials 2017;9:775–781.
17. Agarwal V, Lal P, Pruthi V. Effect of plant oils on *Candida albicans*. Journal of Microbiology, Immunology and Infection 2015;43:447–451.
18. Inouye S, Takahashi M, Abe S. Inhibitory activity of hydrosols, herbal teas and related essential oils against filament formation and the growth of *Candida albicans*. Nihon Ishinkin Gakkai Zasshi 2009;50:243–251.
19. Stevic T, Berić T, Šavikin K, et al. Antifungal activity of selected essential oils against fungi isolated from medicinal plant. Industrial Crops and Products 2014;55:116–122.
20. Kazemi M. Chemical composition and antimicrobial activity of essential oil of *Matricaria recutita*. International Journal of Food Properties 2015;18:1784–1792.
21. Pauli A. α-Bisabolol from chamomile: a specific ergosterol biosynthesis inhibitor. International Journal of Aromatherapy 2006;16:21–25.
22. Matsuoka H, Oh K-B. Compound to regulate the morphological transition of dimorphic Candida. United States Patent Number 2001;6,271,267.
23. Abdallah EM, Qureshi KA, Ali AMH et al. Evaluation of some biological properties of *Saussurea costus* crude root extract. Bioscience Biotech Research Communications 2017;10:601–611.
24. Barrero AF, Oltra JE, Alvarez M, et al. New sources and antifungal activity of sesquiterpene lactones. Fitoterapia 2000;71:60–64.
25. Gwari G, Bhandari U, Andola HC, et al. Volatile constituents of *Saussurea costus* roots cultivated in Uttarakhand Himalayas, India. Pharmacognosy Research 2013;5:179–182.
26. Khan S, Imran M, Imran M, et al. Antimicrobial activity of various ethanolic plant extracts against pathogenic multi drug resistant Candida spp. Bioinformation 2017;13:67–72.
27. Riasat M, Jafari AA, Bahmanzadegan A, et al. The constituents of essential oil in leaves of Karaj accession of Trigonella foenum graecum. National Product Research 2017;31:1709–1712.
28. Braga PC, Alfieri M, Culici M, et al. Inhibitory activity of thymol against the formation and viability of *Candida albicans* hyphae. Mycoses 2007;50:502–506.
29. Ljaljević-Grbić M, Unković N, Dimkić I, et al. Frankincense and myrrh essential oils and burn incense fume against microinhabitants of sacral ambients. Wisdom of the ancients? Journal of Ethnopharmacology 2018;219:1–14.
30. Murakami C, Cordeiro I, Scotti MT, et al. Chemical composition, antifungal and antioxidant activities of *Hedyosmum brasiliense* Mart. ex Miq. (Chloranthaceae). Medicines (Basel) 2017;4:pii:E55. doi:10.3390/medicines4030055.
31. Chen C, Long L, Zhang F, et al. Antifungal activity, main active components and mechanism of *Curcuma longa* extract against *Fusarium graminearum.* PLoS ONE 2018;13:e0194284.
32. Stanciuc AM, Gaspar A, Moldovan L, et al. In vitro antimicrobial activity of Romanian medicinal plants hydroalcoholic extracts on planktonic and adhered cells. Roumanian Archives of Microbiology and Immunology 2011;70:11–14.

33. Baser KH. Biological and pharmacological activities of carvacrol and carvacrol bearing essential oils. Current Pharmaceutical Design 2011;14:3106–3119.
34. Gül S, Demirci B, Baser KH, et al. Chemical composition and in vitro cytotoxic, genotoxic effects of essential oil from *Urtica dioica* L. Bulletin of Environmental Contamination and Toxicology 2012;88:666–671.
35. Rao A, Zhang Y, Muend S, et al. Mechanism of antifungal activity of terpenoid phenols resembles calcium stress and inhibition of the TOR pathway. Antimicrobial Agents in Chemotherapy 2012;54:5062–5069.
36. Monteoliva L, Martinez-Lopez R, Pitarch A, et al. Quantitative proteome and acidic subproteome profiling of *Candida albicans* yeast-to-hypha transition. Journal of Proteome Research 2011;10:502–517.
37. Ayub MA, Hanif MA, Sarfraz RA, et al. Biological activity of *Boswellia serrata* Roxb. oleo gum resin essential oil: effects of extraction by supercritical carbon dioxide and traditional methods. International Journal of Food Properties 2018;21:808–820.
38. Andrews RE, Parks LW, Spence KD. Some effects of douglas fir terpenes on certain microorganisms. Applied and Environmental Microbiology 2018;40:301–304.
39. de Castro PA, Bom VL, Brown NA, et al. Identification of the cell targets important for propolis-induced cell death in *Candida albicans*. Fungal Genetics and Biology 2013;60:74–86.
40. Ioshida MDM, Young MCM, Lago JHG. Chemical composition and antifungal activity of essential oil from Brazilian Propolis. Journal of Essential Oil Bearing Plants 2010;Pl 13:633–637.
41. Rivas da Silva AC, Lopes PM, Barros de Azevedo MM, et al. Biological activities of α-pinene and β-pinene enantiomers. Molecules 2012;17:6305–6316.
42. LaFleur MD, Qi Q, Lewis K. Patients with long-term oral carriage harbor high-persister mutants of *Candida albicans*. Antimicrobial Agents in Chemotherapy 2010;54:39–44.
43. Bonhomme J, d'Enfert C. *Candida albicans* biofilms: building a heterogeneous, drug-tolerant environment. Current Opinions in Microbiology 2013;16:1–6.

Comparison of the effect of inhalation aromatherapy with rose and metoclopramide on anxiety and depression in women with pregnancy nausea and vomiting: a clinical trial

Maliheh Afiat[1], Seyede Houra mosavi Vahed[1], Bahare mohammadipour yazdi[2], Masumeh Ghazanfarpour[3]*, Mahnaz Boroumand Rezazadeh[4]

[1]Women's Health Research Center, Department of Obstetrics and Gynecology, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

[2]Student Research Committee, Mashhad University of Medical Sciences, Mashhad, Iran

[3]Student Research Committee, Kerman University of Medical Sciences, Kerman, Iran

[4]Assistant Professor of Obstetrics and Gynecology Department of Obstetrics and Gynecology, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

Contact information
Masumeh Ghazanfarpour
Email masumeh.ghazanfarpour@yahoo.com

Keywords Rosa damascene, metoclopramide, sleep, nausea, pregnancy

For referencing Afiat M et al. Comparison of the effect of inhalation aromatherapy with rose and metoclopramide on anxiety and depression in women with pregnancy nausea and vomiting: a clinical trial. The Australian Journal of Herbal and Naturopathic Medicine 2021;33(4):12-17

DOI https://doi.org/10.33235/ajhnm.33.4.12-17

Abstract

Objective In light of the importance of using safe drugs during pregnancy, this study was conducted to compare the effect of aromatherapy with rose and metoclopramide on pregnancy-induced depression.

Methods and materials In this cross-over study, subjects consisted of 40 patients with nausea and vomiting who were assigned to two groups (n=20 in each group). The first group was treated with drops of rose essential oil and placebo for 5 days and the second group received drops of placebo essential oil and metoclopramide. Then the drugs were swapped between the two groups and the treatment continued for another 5 days. The Hospital Anxiety and Depression Scale (HADS) was utilised to measure mood swings.

Results The mean age of patients was 22.07±4.94 years. As for the patients' mood swings, the mean score of the HADS was not significantly different between the two groups at the baseline (p=0.82). However, by the end of the 5th day, the score dropped significantly in group A (aromatherapy with rose + placebo) compared to group B (metoclopramide + rose) in terms of anxiety (p=0.008) and depression (p=0.009). However, a significant difference was observed between both groups on the 10th day in both anxiety (p=0.19) and depression (p=0.49).

Conclusion As suggested by the results of this study, the positive effects of aromatherapy with rose eclipse those of metoclopramide on mood swings (both anxiety and depression) in patients with pregnancy nausea and vomiting.

Introduction

Pregnancy nausea and vomiting are common in early pregnancy; they are reported in 50–80% of pregnant women[1]. Pregnancy nausea and vomiting usually begin 6–8 weeks after the 1st day of the last menstruation and continue until week 16. In 90% of cases, they disappear by the 22nd week[2]. If pregnancy nausea and vomiting are severe and lasting, they may lead to hyperemesis gravidarum disease which causes maternal weight loss, severe dehydration, electrolyte imbalance, and ketone excretion in the urine. Research has shown that lasting nausea and vomiting are associated with intrauterine growth restriction (IUGR). Pregnancy nausea and vomiting produce adverse physical and mental effects[3].

Arsenault investigated the complications of pregnancy nausea and vomiting along with their socio-economic effects, mainly because it is a leading cause of pregnant women's absence from work[4]. However, the drugs used to control nausea and vomiting in pregnancy have adverse effects; diphenhydramine and phenothiazines are associated with a higher rate of cleft lip and cleft palate as well as structural abnormalities in infants[5].

Therefore, non-pharmacological methods have been developed to prevent and treat nausea and vomiting; it is highly important to find more effective and less complicated treatments to help pregnant women with nausea and vomiting during pregnancy. As a form of complementary medicine, aromatherapy represents a non-pharmacological traditional method that is based on distilled plant essential oils. The aromatherapy's mechanism of action is through the olfactory system and the skin. Today, aromatherapy embraces approximately 40 different types of oils, either alone or in combination. However, the aromas of basil, thyme, lily, peppermint, rosemary and juniper can harm the foetus and their internal and external application may induce abortion[6].

Rose contains ingredients such as Geraniol, Citronellol, Linalol and Stearoptene, and this herbal medicine is often utilised to mitigate stress, cure depression and chronic insomnia, and induce sedative, analgesic, anticonvulsant and skin repair effects[2]. The findings of animal studies show that rose produces anti-anxiety[2] and anti-depression[7] effects, and human studies have confirmed its anti-anxiety effects[8,9]. Considering its psychological effects on alleviating anxiety, depression and fear, it is recommended for women with pregnancy nausea and vomiting. The goal of this study is to compare the effect of aromatherapy with rose and metoclopramide on anxiety and depression in women with pregnancy nausea and vomiting.

Materials and method

This is a randomised cross-over clinical trial with a placebo. This study was conducted in 2017 in the special clinic of Imam Reza Hospital affiliated with Mashhad University of Medical Sciences in Iran. Given the paucity of studies on this subject in Iran and other countries, first, a pilot study was conducted on 20 people in each group. Participants consisted of primigravida with intended pregnancies referring to the special clinic of Imam Reza Hospital who complained of nausea and vomiting between 7–14 weeks of pregnancy and who were diagnosed with mild to moderate nausea and vomiting by a gynaecologist.

Patients filled out an informed consent form before the study. The research was also approved by the ethics committee of Mashhad University of Medical Sciences (ethics code IR.Mums.fm.Rec.1395.88) and conducted in accordance with the principles of the Helsinki Declaration in 2016. This study has been registered in the Iranian Registry of Clinical Trials (IRCT20170628034792N4). At the outset of the study, the method and goal of the research were fully and clearly explained to the participants and written informed consent was obtained from all individuals.

Inclusion criteria

Primigravida with a singleton and intended pregnancy in their 7–14 weeks of pregnancy who had a mild and moderate craving, and their daily nausea and vomiting had not improved with normal diets and instructions.

Exclusion criteria

An underlying illness that require medication, hepatitis, gastritis, increased cranial pressure and pancreatitis, and pregnancy complications including the risk of abortion, bleeding and pyelonephritis.

Data collection tools

In this study, the patient data was collected by a checklist that recorded information on age, gestational age, body mass index (BMI) and level of education. The Hospital Anxiety and Depression Scale (HADS) was used to measure patients' mood swings. This tool consists of 14 items, of which seven assess depression and seven measure anxiety. Each item is rated on a 4-point Likert scale and its total score is between 0–3[10]. The estimated Cronbach's alpha of this scale is 0.78 in Iran[11], 0.88 in Greece[12] and 0.84 in Sweden[13].

Study design

Participants were assigned to two groups using the permuted block randomisation method. Each patient was randomly assigned to one of the two treatment groups by a colleague who was not part of the research team. In group A, patients were treated with rose aromatherapy + placebo in the first 5 days and metoclopramide + placebo in the second 5 days. Conversely, in group B, patients were first treated with metoclopramide + placebo in the first 5 days and then with rose aromatherapy + placebo in the second 5 days. Patients were examined three times (at the beginning of the study, at the end of the first 5 days, and the end of the 10th day) by a questionnaire.

In group A, 20 patients were instructed to pour two drops of rose essential oil on a handkerchief during the fits of nausea and vomiting, hold it 3cm away from the nose and take a deep breath through the nose for 3 seconds. They were also instructed to use 15 drops of the placebo three times a day. In group B, 20 patients were instructed to use metoclopramide (15 drops three times a day at 6am, 1pm, and 9pm) and placebo essential oil during nausea. After 5 days, the drugs were swapped so that group A received drops of metoclopramide and placebo essential oil and group B received drops of rose essential oil and placebo.

Placebo essential oil, resembling rose essential oil both in shape and colour, was made by Barij Essence Company. In this study, blinding was ensured by the company and the codes were disclosed only at the end of the study. The double blinding was ensured by coding so that the pharmaceutical company coded boxes according to a random list before delivering them to the researchers. The

content of each box was revealed to researchers at the end of the research when the coded boxes were opened.

Essential oil preparation method

Rosewater and rose essential oil are extracted from a species of rose family called Damask rose (scientific name: *Rosa damascene*). In this process, the petals of the flower are distilled by water vapour. The product amassing at the top of the tower, which is rich in rose extract, is split into two oily and aqueous phases. The former is called the primary essential oil and the latter is known as rosewater grade 1.

Typically, the compounds in the primary essential oil are heavy and not suitable for medicinal purposes. Therefore, to extract high-quality essential oil rosewater, grade 1 is re-distilled. The outcome of this distillation is three new products called rosewater grade 2, rose water grade 3 and the secondary essential oil. Rosewater grade 3 is the solution remaining at the bottom of the distillation tower. The biphasic division of products at the top of the tower produces secondary essential oil and rosewater grade 2. Secondary essential oils have wide applications in the pharmaceutical industry. Table 1 shows the volume of rose products.

According to Table 1, this compound makes up for 2wt% of rose water grade 1. However, as shown in Table 1, there are other compounds in essential oil grade 2 such as geranium, citronellol, etc. Therefore, an important issue concerning rose water is its concentration and production of essential oil with enhanced medicinal properties[14].

Statistical analysis

The collected data was entered in SPSS 20 software. First, the Kolmogorov-Smirnov test was carried out to assess the normal distribution of data. For the comparison of quantitative variables between the two groups, t-test and Mann-Whitney-U were applied for data with normal and abnormal distribution, respectively. Paired samples t-test and Wilcoxon test were also used to compare pre- and post-intervention variables for data with normal and abnormal distribution, respectively. p value <0.05 was considered statistically significant.

Results

The clinical and demographical characteristics of the two groups at the baseline are compared in Table 2. The two groups were almost identical in clinical and demographical characteristics except for level of education (p=0.04)

In this study, three standard questionnaires were used to compare patients' responses to treatment. The analysis of patients' mood swings using HADS suggested that the mean score of the questionnaire in group A (11.75±11.27) and group B (11.35 ± 9.7) was not significantly different

Table 1. Percentage of compounds in rose products (based on GC-MS in Barij Essential Oil Research Laboratories)

Ingredients	Primary essential oil	Secondary essential oil	Rosewater grade 1	Rosewater grade 2	Rosewater grade 3
2-phenylethanol	0.60	1.73	84.22	91.49	94.71
Citral	–	0.81	–	–	–
Citronellol	20.59	47.35	2.9	1.60	–
Eugenol	0.36	1.54	1.55	2.33	–
Gerantol	8.19	22.57	2.52	1.95	–
Geranyl acetate	.210	1.65	–	–	–
Germacrene D	2.54	0.27	–	–	–
Hexadecane–1–ol	8.29	2.14	0.49	–	–
Linalool	1.12	5.28	0.66	–	–
Methyl eugeno	0.60	1.88	–	–	–
Myrcene	0.83	–	–	–	–
Nerol	–	0.50	–	–	–
n-Heptadecane	5.93	1.46	1.27	–	2.11
n-Nonadecane	42.40	10.78	3.30	–	–
o-Cresol	–	–	2.77	2.62	3.18
Pentadecane	0.88	0.14	–	–	–
Terpinene-4-ol	–	0.27	–	–	–
α-guatene	0.88	0.14	–	–	–
α-humulene	0.78	0.13	–	–	–
α-Pinene	2.24	–	–	–	–
α-terpineol	–	1.08	0.39	–	–
-caryophylleneβ	1.28	0.20	–	–	–
-pineneβ	0.39	–	–	–	–

between the two groups at the baseline (p=0.82). By the end of the 5th day, in group A (rose aromatherapy + placebo), this score dropped from 11.75±11.27 to 5.25±4.5, but in group B (metoclopramide + rose), this score declined slightly from 11.35±9.7 to 11.25±8.12. At this stage, there was a significant difference between the two groups in terms of HADS score, as measured by the Mann-Whitney test (p=0.008).

The cross-over analysis of treatment type in the second 5 days showed that the score of the HADS questionnaire in group A (metoclopramide + placebo) increased to 10.5+8.23 by the end of the 10th day, but in group B (rose aromatherapy + placebo), it decreased to 8.35±8.24. The results of the Mann-Whitney test showed that this difference was not significant between the two groups at this stage (p=0.192).

The within-group analysis of changes revealed that the HADS anxiety score in group A decreased significantly on the 5th day versus the 1st day (p<0.001), but again spiked dramatically on the 10th day versus the 5th day (p<0.001) (Table 3). However, in group B, HADS scores did not alter significantly on the 5th day in comparison to the baseline (p=0.754), but they significantly declined on the 10th day as opposed to the 5th day (p=0.007).

Also, the analysis of seven items related to the assessment of depression in the HADS exhibited that, at the baseline, the mean score of the questionnaire was 4.95±5.6 in group A and 5.3±4.53 in group B, and there was no significant difference between the two groups in this regard (p=0.758). By the end of the 5th day, this score dropped from 4.95±5.6 to 2.25±2.84 in group A, but in group B (metoclopramide + rose), this score slightly fell from 5.2±4.53 to 9.7 to 5.1±3.83. At this stage, there was a significant difference between the two groups in terms of HADS score, as measured by the Mann-Whitney test (p=0.008).

The cross-over study of the treatment type in the second 5 days showed that the depression score of HADS in group A (metoclopramide + placebo) increased to 4.35±3.85 by the end of the 10th day. In group B (rose aromatherapy + placebo), however, it dropped to 3.9±3.93 by the end of the 10th day. The results of the Mann-Whitney test showed that the two groups were not significantly different at this stage (p=0.492).

The within-group analysis of changes showed that the depression score of HADS items in group A fell significantly on the 5th day in comparison to the baseline (p=0.002), but it improved significantly on the 10th day as opposed to the 5th day (p=0.01). However, in group B, HADS scores did not change significantly on the 5th day in comparison to the baseline (p=0.776), though a significant reduction was observed on the 10th day compared to the 5th day (p=0.017) (Table 4).

Discussion

As suggested by the review of literature, this is the first study to compare patients' mood swings in two intervention groups treated by rose aromatherapy + placebo and metoclopramide + rose. The analysis of patients' mood swings indicated that, by the end of

Table 2. Comparison of clinical and demographical characteristics of the two groups at the baseline

Characteristic	Group A n=20	Group B n=20	p value
Clinical characteristics			
Age (mean)	22.15±4.15	22±5.73	0.925
Gestational age	11.3±2.86	10.7±2	0.448
Body Mass Index (BMI)	23.73±3.1	22.67±3.15	0.294
Level of education			
Primary school	3(15%)	9(45%)	0.04
Diploma or Associate's degree	11(55%)	9(45%)	
Bachelor's degree	6(30%)	2(10%)	

Table 3. Comparison of the anxiety scores obtained from the HADS questionnaire in the two groups at the baseline, day 5 and day 10 along with the within-group analysis of changes

Characteristic	Group A n=20	Group B n=20	p value
Anxiety score of HADS at the baseline	11.75±11.27	11.35±9.7	0.82
Anxiety score of HADS on day 5	5.25±4.5	11.25±8.12	0.008
Anxiety score of HADS on day 10	10.5±8.23	8.35±8.24	0.192
p value (day 5 vs. baseline)	<0.001	0.754	
p value (day 10 vs. day 5)	<0.001	0.007	

* Wilcoxon signed-ranked test was used to compare the two groups.

Table 4. Comparison of the depression scores obtained from the HADS questionnaire in the two groups at the baseline, day 5 and day 10 along with the within-group analysis of changes

Characteristic	Group A n=20	Group B n=20	p value
Depression score of HADS at the baseline	4.95±5.6	5.2±4.53	0.758
Depression score of HADS score on day 5	2.25±2.84	5.1±3.83	0.009
Depression score of HADS on day 10	4.35±3.85	3.9±3.93	0.492
p value (day 5 vs. baseline)	0.002	0.776	
p value (day 10 vs. day 5)	0.01	0.017	

* Wilcoxon signed-ranked test was used to compare the two groups.

the 5th day, patient scores were significantly lower in group A (rose aromatherapy + placebo) than in group B (metoclopramide + rose). The cross-over analysis of the type of treatment in the second 5 days did not reveal a significant difference between the two groups at this stage.

In a 2011 animal study by Rezaei et al.[2], researchers investigated the anti-anxiety and pre-anaesthetic effects of rose extract versus diazepam in male Wistar rats. In this study, the elevated plus maze and Rotarod were used to measure the anti-anxiety effects of drugs. They found that rose extract significantly increased the duration of ketamine-induced sleep. The results of this study, aligned with the current research, demonstrate the anti-anxiety and sedative effects of rose extract[2].

In 2015, Ataullahi et al.[8] investigated the effect of rose essential oil on primary dysmenorrhea and its associated systemic symptoms. The findings of this study revealed that the severity of dysmenorrhea and its associated systemic symptoms was significantly lower in the group receiving rose essential oil than in the control group. The analysis of specific patients' symptoms, consistent with the findings of the present study, showed a significant reduction in the severity of nausea and vomiting ($p=0.016$) as well as the severity of mood swings ($p<0.001$) in participants with dysmenorrhea that had received rose essential oil[8].

The positive effects of rose can be attributed to the compounds and ingredients of this substance, including flavonoids. Like benzodiazepines, flavonoids bind to gamma-aminobutyric acid receptors (GABA) in the central nervous system (CNS) and open the chlorine channel which induces a relaxing and muscle-relaxing effect[15]. Rose has a positive effect on the CNS. Previous studies, in line with ours, have reported the significant positive effects of this substance on both anxiety disorders and depression. A 2002 study by Umezu et al.[16] stated that, out of nine major ingredients of rose extract, citronellol and 2-phenyl ethyl alcohol had remarkable anti-anxiety effects. Boskabadi et al.[17] also reported that the flavonoids in roses produced hypnotic and anti-anxiety effects.

The results of a 2000 study by Bums et al.[9] on 8,085 cases of delivery showed that rose aromatherapy mitigated anxiety by 71% in women in labour. In addition, only 14% of these women required local anaesthesia, and pethidine intake dropped from 6–0.2% in them. Regarding the antidepressant effects of this substance, previous research aligned with this paper has observed the positive antidepressant effects of rose[7,17]. In this respect, Zarghami et al.[7] compared the effects of rose with placebo and amphetamines in a laboratory study on rats. Their findings revealed that rose drops at concentrations of 10, 20 and 40% significantly mitigated the severity of depression compared to the placebo group which was analogous to amphetamines[7].

Another important contribution of this study is the adoption of valid and reliable questionnaires to assess the severity of nausea and vomiting along with the severity of depression and anxiety. One major limitation of this study is its small sample size. As a pilot study, it only consisted of 40 pregnant women which can undermine the validity and generalisability of the results. Hence, it is recommended to compare the effect of this substance with other standard treatments for nausea and vomiting or to investigate its synergistic effect in combination with those drugs. Moreover, further longitudinal studies are recommended to examine the long-term effects and possible side effects of this treatment. The limitations of a cross-over design may pose a challenge to ensuring blinding as well as maintaining the period effect, the sequence effect, and the treatment effect.

Conclusion

The results of this study demonstrated that the positive effects of rose aromatherapy on mood swings (both anxiety and depression) were significantly higher than metoclopramide in patients with pregnancy nausea and vomiting.

Conflict of interest

The authors declare no conflicts of interest.

Funding

The authors received no funding for this study.

References

1. Smith C, Crowther C, Beilby J. Acupuncture to treat nausea and vomiting in early pregnancy: a randomized controlled trial. Birth 2002;29(1):1–9.
2. Rezaie A, Mosavi G, Ahmadizadeh C, Jafari B. Study of sedative, preanaesthetic and anti-anxiety effects of Rosa damascene herbal extract in comparison with diazepam in rat. Tehran University Medical Journal 2011;69(3).
3. Niebyl JR. Nausea and vomiting in pregnancy. New England Journal of Medicine 2010;363(16):1544–50.
4. Arsenault M-Y, Lane CA, MacKinnon CJ, Bartellas E, Cargill YM, Klein MC, et al. The management of nausea and vomiting of pregnancy. Journal of Obstetrics and Gynaecology Canada: JOGC (Journal d'obstetrique et gynecologie du Canada) 2002;24(10):817–31; quiz 32.
5. Carranza-Lira S, Gooch A, Saldivar N, Osterwalder MS. Climacteric symptom control after the addition of low-dose esterified conjugated estrogens to raloxifene standard doses. International Journal of Fertility and Women's Medicine 2007;52(2–3):93.
6. Fuh J, Wang GC. Aromatherapy for pregnancy and birth. International Journal of Childbirth of Childbirth Education 2001;16(3):18–9.
7. Zarghami M, Farzin D, Bagheri K. Anti depressant effects of Rosa Damascena on laboratory rats: a controlled experimental blind study. Journal of Mazandaran University of Medical Sciences 2001;11(33):27–33.
8. Ataollahi M, Akbari SA, Mojab F, Roshanaie G. Effects of aromatherapy by Rosaceous on the severity and systemic symptoms of primary dysmenorrhea. Advances in Nursing & Midwifery 2015;25(89):59–67.
9. Burns EE, Blamey C, Ersser SJ, Barnetson L, Lloyd AJ. An investigation into the use of aromatherapy in intrapartum midwifery practice. The Journal of Alternative and Complementary Medicine 2000;6(2):141–7.
10. Zigmond AS, Snaith RP. The hospital anxiety and depression scale. Acta Psychiatrica Scandinavica 1983;67(6):361–70.
11. Montazeri A, Vahdaninia M, Ebrahimi M, Jarvandi S. The Hospital Anxiety and Depression Scale (HADS): translation and validation study of the Iranian version. Health and Quality of Life Outcomes 2003;1(1):14.
12. Michopoulos I, Douzenis A, Kalkavoura C, Christodoulou C, Michalopoulou P, Kalemi G, et al. Hospital Anxiety and Depression Scale (HADS): validation in a Greek general hospital sample. Annals of General Psychiatry 2008;7(1):4.
13. Lisspers J, Nygren A, Söderman E. Hospital Anxiety and Depression Scale (HAD): some psychometric data for a Swedish sample. Acta Psychiatrica Scandinavica 1997;96(4):281–6.
14. Babaei-Ravandi B, Roustaazad R, Soltanieh M. Estimating the flux and composition of the Rosa damascena essences pervaporated from the rose extract. 2006.
15. Latthe P, Latthe M, Say L, Gülmezoglu M, Khan KS. WHO systematic review of prevalence of chronic pelvic pain: a neglected reproductive health morbidity. BMC Public Health 2006;6(1):1–7.
16. Umezu T, Ito H, Nagano K, Yamakoshi M, Oouchi H, Sakaniwa M, et al. Anticonflict effects of rose oil and identification of its active constituents. Life Sciences 2002;72(1):91–102.
17. Boskabady MH, Shafei MN, Saberi Z, Amini S. Pharmacological effects of Rosa damascena. Iranian Journal of Basic Medical Sciences 2011;14(4):295.

Australian Journal of Herbal and Naturopathic Medicine **2022** 34(1)

Medicinal mushrooms (specifically *Ganoderma lucidum or* Reishi) as an adjuvant treatment in breast cancer

Catherine Lilburn (UTas) BMedMmentProfHonsCM
27 Wilkies Street, Bulli, NSW 2516, Australia
Email cathlilburn@me.com

Keywords medicinal mushrooms, *Ganoderma lucidum*, Reishi, breast cancer, cancer-related fatigue

For referencing Lilburn C. Medicinal mushrooms (specifically *Ganoderma lucidum* or Reishi) as an adjuvant treatment in breast cancer. The Australian Journal of Herbal and Naturopathic Medicine 2022;34(1):18-23

DOI https://doi.org/10.33235/ajhnm.34.1.18-23

Submitted 16 September 2021, Accepted 2 December 2021

Abstract

Medicinal mushrooms have been used in the traditional medicines of Asia, in particular China and Japan, for centuries. Medicinal mushrooms include some well-known species – *Ganoderma lucidum* (Reishi), *Trametes/Coriolus versicolor* (Turkey tail) and *Lentinula edodes* (Shiitake). There has been a significant increase in medicinal mushroom research in the past few decades, and particularly in relation to cancer. Breast cancer is the most common cancer diagnosis for women and although treatment outcomes have improved life expectancy, there is a need to improve the quality of life (QoL) impacts of oncology treatment, especially cancer-related fatigue (CRF) and immunosuppression. In preparation for this review, a search was conducted of PubMed, Google Scholar and the Cochrane library for original research on medicinal mushrooms (and *G. lucidum* in particular) in relation to breast cancer. This paper overviews the in vivo, in vitro and limited clinical research that has been conducted on breast cancer and *G. lucidum* (Reishi mushroom). The in vivo and in vitro research has demonstrated reductions in breast cancer cell growth and metastasis, which is encouraging. Systematic reviews of *G. lucidum* and cancer have also proved promising. Considering these results and given the popularity of complementary medicine (CM) as an adjunct breast cancer treatment, future high quality clinical trials that investigate the efficacy of *G. lucidum* in supporting the QoL in women confronted with a breast cancer diagnosis are warranted.

Background

Medicinal mushrooms are a type of medicinal plant or macroscopic funghi[1]. They are members of the Basidiomycetes class of mushrooms and have been valued around the world for their nutritious content[2]. Medicinal mushrooms have also been used for centuries in the traditional medicines of Asia, in particular China and Japan[3]. There are approximately 10,000 different species of mushrooms and estimations of up to 200 having medicinal actions[4]. Edible medicinal mushrooms species include species of *Lentinula* and *Grifola*, whereas inedible species regarded for their medicinal effects include *Ganoderma* and *Trametes (Coriolus)*[2]. Medicinal mushrooms are thought to possess around 130 medicinal functions[1]. The biological activity of the mushrooms is found in the fruiting body, the cultured mycelia and the cultured broth[1]. Traditionally these parts of the mushrooms were dried and used as medicine.

Contemporary context of studies into medicinal mushrooms

The past 60 years has seen an exponential rise in research into medicinal mushrooms in a range of health conditions[5]. The research has focussed on the use of medicinal mushrooms in a diverse range of health conditions ranging from diabetes[6,7] to cancer[8,9]. This is because there are a multitude of pharmacological actions that medicinal mushrooms have on the human body, including antimicrobial, cytotoxic, antidiabetic, antioxidant, anticancer, hepatoprotective, immunomodulatory and prebiotic[10]. Modern medicinal mushrooms are prepared into dietary supplements in a number of ways using artificially cultivated fruit body powders, dried preparations that combine the substrate and mycelium, dried fruit body made into tablets or capsules, and mushroom spores[1].

Medicinal mushrooms and cancer research

Cancer is the second leading cause of death in industrialised countries after cardiovascular disease[11]. The treatments utilised by conventional medicine include surgery, chemotherapy, radiation, immunotherapies and hormonal therapies[11]. Despite advancements in cancer treatment, significant issues persist[12]. There are a number of side effects that can be associated with cancer treatments such as fatigue, immunosuppression, gastrointestinal symptoms, and skin and mucous membrane irritations[11].

Cancer-related fatigue (CRF) is one of the most common experiences that patients experience after oncology treatment[13,14], impacting 59–100% of patients[15]. However, CRF is a complex and multi-factorial condition that remains poorly understood and undertreated[14,16]. There are many possible causes of the condition and underlying pathophysiology potentially involved, including cytokine dysregulation and HPA (hypothalamic-pituitary-adrenal) axis disruption[16]. CRF is distressing and impacts greatly on a patient's quality of life (QoL)[16]. This has led to patients investigating complementary medical (CM) treatments to help reduce the side effects associated with conventional cancer treatment and to improve their health-related outcomes.

There are a number of medicinal mushrooms that have been studied more extensively for their CM role, primarily as immunological support and, to a lesser extent, as QoL support. The bioactive metabolites found in medicinal mushrooms have been shown in studies to be potential biological immunotherapeutic agents that can stimulate the immune system against cancer cells[17]. *Ganoderma lucidum* (Reishi or *Lingzhi*) and *Coriolus versicolor* (Turkey tail or *Yunzhi*) are two commonly used and often studied medicinal mushrooms in adjuvant cancer treatment. A recent systematic review of these two mushrooms outlined the biological activity of *C. versicolor*, which includes polysaccharide krestin (PSK) and polysaccharide peptide (PSP), and the activity of *G. lucidum,* which contains beta-glucans and triterpines, all of which have selective cytotoxic activity against tumour cells[18]. An earlier systematic review of *C. versicolor* for lung cancer found that PSK might improve immune function, reduce tumour-associated symptoms, and extend survival time for patients[19].

Mushrooms and breast cancer

Dietary intake of mushrooms and reduced breast cancer risk

There have been a number of studies completed that link the consumption of mushrooms with a reduced risk of breast cancer. A 2008 Korean case-control study concluded that dietary intake of mushrooms may reduce the risk of breast cancer in postmenopausal women[20]. In contrast, a Korean study published in 2010, showed a reduced risk of breast cancers and in particular hormonal receptor positive cancers in premenopausal women who consumed mushrooms regularly in their diet, but not postmenopausal women[21], while a 2009 Chinese case-control study of 1009 women showed a dose dependent inverse relationship between the risk of breast cancer in both premenopausal and menopausal women[22]. A 2021 systematic review and meta-analysis of observational studies concluded that higher consumption of mushrooms was associated with a low risk of cancer, especially breast cancer[23].

Research on medicinal mushrooms and breast cancer

Female breast cancer is now the most commonly diagnosed form of cancer in the world, accounting for 11.7% of new cases in 2020[24]. It is also the leading cause of cancer deaths in women globally[24]. There have been improvements in survival rates of women diagnosed with breast cancer in Western countries, largely due to earlier detection rates[25]. However, women treated for breast cancer with a combination of surgery, chemotherapy, radiation and hormonal therapies can experience a range of side effects including fatigue, depression, decreased aerobic capacity, weight gain and decreased QoL[26]. This highlights the importance of finding effective support for women to manage long-term consequences of breast cancer treatments and improve their QoL[25].

A 2015 systematic review on the topic found that up to 80% of breast cancer patients used CM throughout their breast cancer treatment[27]. Breast cancer patients are choosing CM to potentially improve their QoL post-cancer treatment and present cost-effective and supportive strategies[28]. Medicinal mushrooms are one type of CM used by women through their conventional oncology breast cancer treatment and afterwards. Therefore, it is important to investigate the research that has been conducted on medicinal mushrooms in relation to breast cancer. Research has focussed on specific medicinal mushrooms or combinations of them, but this paper will focus on research into *G. lucidum* (Reishi) to simplify this process as there is a large amount of in vivo and in vitro research conducted on medicinal mushrooms and cancer. In contrast, there have been very few clinical human trials completed on the topic.

Research on *G. lucidum* (Reishi) and breast cancer

G. lucidum has been regarded in traditional Chinese medicine (TCM) as a mushroom of immortality that can promote health and extend life[29]. The scientific research on Reishi mushrooms and breast cancer has mostly focussed on the biologically active compounds found in *G. lucidum* that have immunological anti-cancer effects[30]. These compounds include the triterpenoids (ganoderic acids and ganoderic alcohols) and polysaccharides (mainly glucans such as beta-D-glucans and glycoproteins)[30]. However, over 240 secondary compounds have been isolated from *G. lucidum*[31]. This makes the research more complex in nature due to the potential large number of metabolites that can be investigated. This paper will summarise the immunological research that has been conducted on *G. lucidum* and then move onto CRF research.

Immunological research

In vitro research

The in vitro research conducted on Reishi and breast cancer cells has investigated the action of biological compounds on various immunological pathways (for example NF-kappaB and mTOR signalling) involved with tumour cell growth and metastatic progression of breast cancer cells. A 2002 in vitro study showed that an alcohol extract of *G. lucidum* had an inhibitory effect on breast cancer cell proliferation in a dose dependent manner[32]. In the same year a study found that spores from *G. lucidum* inhibited the invasiveness of breast and prostate cancer cells[33]. Since then, a number of research groups have used ganoderic acids (found in *G. lucidum*) to show that it suppresses growth and invasive behaviours of breast cancer cells[34]; suppressed growth and angiogenesis of breast cancer cells via modulating the NF-kappaB signalling pathway[35]; and induced DNA damage and apoptosis of breast cancer cells[36].

In 2011, Martinez-Montemayor et al.[37] conducted a trial on the effects of *G. lucidum* on inflammatory breast cancer cells which are found in advanced breast cancer patients. The study showed that Reishi reduced the expression of genes involved in cancer cell survival and proliferation and in cancer cell invasion and metastasis[37]. The same study group published the results of an investigation 2 years later that demonstrated that Reishi mushrooms downregulated the mTOR pathway which is involved in tumour growth, angiogenesis and metastasis in vitro[38].

More recent studies include a trial that demonstrated that *G. lucidum* suppressed the proliferation and migration of breast cancer cells by inhibiting gene expression[39]. In 2019 Acevedo-Diaz et al.[40] published the results of an in vitro study investigating the impact that *G. lucidum* had on the motility of breast cancer cells. The authors found that Reishi reduced the cellular activity and downregulated signalling molecules involved with breast cancer metastasis[40]. Further research was published in 2020 showing the impact of *G. lucidum* spore oil on both breast cancer cells and on tumours in vivo[41]. The study showed that Reishi spore oil inhibited the growth of breast cancer cells and reduced the growth of tumours by inducing apoptosis[41].

In vivo research

In vivo research has focussed on the impact of Reishi mushroom on tumour cell growth and metastasis. In 2014, Loganathan et al.[42] published a study which investigated the in vivo effects of *G. lucidum* extract on the growth of breast cancer tumours and on the metastasis from breast to lung and found a statistically significant inhibition of breast–lung metastases in the treatment group. They found this effect was due to the downregulation of particular genes that mediate breast-lung metastases[42].

Another study group showed that immune-compromised mice injected with inflammatory breast cancer cells and then treated with Reishi mushroom for 13 weeks demonstrated a reduced tumour growth and weight of approximately 50%[38]. In 2018, Rios-Fuller et al.[43] reported on the findings of a study that investigated the impact of *G. lucidum* extract on triple negative breast cancer cells in vitro and in vivo (a preclinical animal study). They found that the mushroom extract downregulated the STAT3 pathway which is involved in the progression and development of breast cancer stem cells[43].

More recently, an in vivo study demonstrated improved tumour control and improvements in gut microbiota from using *G. lucidum* spores in conjunction with the chemotherapy agent Paclitaxel in a murine breast cancer model[44]. In 2020, Roda et al.[45] published the results of a trial that used a medicinal mushroom blend (which included *G. lucidum*) in a triple negative mouse breast cancer model. The authors found that the treatment caused a significant decrease in pulmonary metastasis density and a reduction in inflammatory and oxidative stress pathways[45]. They also recommended clinical trials be conducted to translate the results of this trial into a clinical setting[45].

Clinical trials

There is a significant lack of evidence on the benefits of *G. lucidum* for breast cancer patients in a clinical setting. However, in 2021, Deng et al.[46] prospectively investigated the T-lymphocyte, cytokine and inflammatory biomarkers of 120 post-operative lung and breast cancer patients receiving chemotherapy to predict the immunological benefits of *G. lucidum.* The authors found there were immunological benefits (in the T-lymphocyte and cytokine measures and improved neutrophil-to-lymphocyte ratio and albumin-to-globulin ratio) in the treatment group[46].

CRF in breast cancer patients

There has been scant research conducted on the impact of medicinal mushrooms (or Reishi in particular) on CRF. One exception is the Zhao et al.[47] small pilot study of 48 breast cancer patients receiving hormonal therapy showing that *G. lucidum* (Reishi) spores reduced symptoms of CRF in these patients. The study assessed qualitative measures (including energy, fatigue and mood ratings) and they measured a variety of quantitative measurements: TNFα-, IL6, kidney and liver function results at the start and end of the trial after participants consumed Reishi mushroom or a control[47]. The study showed improvements in reporting of energy ratings by participants and reductions in inflammatory marker measures[47]. The authors acknowledged the shortcomings of the trial and the need for more rigorous and larger clinical studies to confirm the efficacy of using Reishi mushrooms to support energy levels in breast cancer patients undergoing hormonal treatments[47]. However, the trial showed some promising clinical results for Reishi and breast cancer patients experiencing CRF.

Additionally, a large cohort study was conducted in China to assess the impact of Reishi mushroom (or ginseng) on breast cancer patients[48]. The authors found

a positive correlation between Reishi mushroom use and social wellbeing scores; however, they found a negative correlation with physical wellbeing scores[48]. This study had contrasting results to various studies showing improved physical frailty ratings from Reishi mushroom treatment in various settings in China[49]. Additionally, the Jin et al.[50] Cochrane review reported relative improvements in the QoL (as measured by the Karnofsky score) of cancer patients taking Reishi mushroom treatment, in contrast to control groups.

Summary of *G. lucidum* cancer research

Systematic reviews of *G. lucidum* and cancer

There have not been any systematic reviews of *G. lucidum* and breast cancer, but there have been a number of relevant systematic reviews completed on *G. lucidum* and cancer. Systematic reviews (and Cochrane reviews in particular) are reliable forms of evidence in evidence-based medicine because of the detailed and rigorous methods employed in the analysis of the relevant clinical trials. Jin et al.[50] published a Cochrane review in 2016 on Reishi and cancer. They found that there was insufficient evidence to justify the use of Reishi as a first line cancer treatment. However, they concluded that Reishi could be used as an alternate adjunct treatment to conventional cancer treatment given its potential benefits for stimulating host immunity and improving tumour response[50].

Zhong et al.[18] conducted a systematic review and meta-analysis of 23 trials involving 4246 cancer patients treated with *G. lucidum* (Reishi) and *C. versicolor* (Turkey tail) as adjuvant cancer therapy. The meta-analysis showed that both mushrooms significantly increased the CD3 and CD4 T helper cell counts effectively reducing the immunosuppression involved with chemotherapy[18]. The authors concluded that both mushrooms may have potential beneficial effects on overall survival and QoL of cancer patients[18].

A systematic review of non-clinical trials involving polysaccharides with anti-tumour effect and their role in breast cancer was published in 2021[51]. The authors concluded that some polysaccharides (including the beta-glucans found in *G. lucidum* and medicinal mushrooms more broadly) deserved more detailed and structured studies of their impact on breast tumours[51]. Another systematic review was published in 2021 which focussed on the immunomodulatory effects of Chinese herbal nedicines (including *G. lucidum*) on natural killer (NK) cell populations for cancer therapy and found a positive correlation with QoL markers[52].

G. lucidum safety

The 2021 Deng et al.[46] prospective study on Reishi use in a clinical setting concluded that there were no serious side effects associated with its use. The Cochrane review of Reishi mushroom and cancer only found one study that reported minor side effects from treatment, but found no reporting of any major haematological or hepatological toxicity[50]. A Cochrane review is considered a reliable form of evidence, so this is a positive finding to support the safe use of Reishi mushroom as an adjuvant cancer treatment.

In addition, Lam et al.[53] published a very relevant systematic review in 2021 of herb–drug interactions between *Lingzhi* (Reishi mushroom) and *Yunzhi* (Turkey tail mushroom) and cytotoxic cancer drugs. The authors found that, from a pharmacodynamic perspective, combining the two medicinal mushrooms with cytotoxic drugs could result in improved survival, QoL and immune modulation, plus a reduction in tumour lesions and chemotherapy associated adverse reactions[53]. However, Lam et al.[53] recommended further studies into pharmacokinetic interactions between medicinal mushrooms and chemotherapy drugs.

Literature limitations

G. lucidum has demonstrated some efficacy as an adjuvant treatment option for breast cancer via the immunological effects of its biologically active compounds, such as beta glucans. However, Sullivan et al.[3] back in 2006 noted that there had been insufficient large, high quality, clinical studies conducted on medicinal mushrooms. This remains the case in 2021. There is a stark gap between the data from preclinical trials and human studies[54]. There have also been some unsuccessful clinical trials conducted on *G. lucidum* in the past decade[55]. This has been partly explained by the complexity of the mushroom composition[55]. Additionally, a sample of *G. lucidum* is influenced by a number of factors including the growing conditions, strains and methods of extraction[56]. This in turn dictates the amount and content of pharmacologically active material in a sample of *G. lucidum*, further complicating the research[56].

This review revealed that there has been limited clinical research conducted on the use of medicinal mushrooms in individual cancer cohorts, especially in a Western context. This could partly be attributed to the high cost of conducting such trials. A 2013 report into the cost of conducting clinical trials in Australia found that it cost on average $6000 per oncology patient to participate in a randomised controlled trial, not including the cost of blood tests, diagnostic tests and overheads[57]. This report is 8 years old, so the costs are likely to be higher now. A 2016 study investigating the costs of a Phase 1 clinical pharmaceutical trial in the US found that the average cost ranged from US$1.4 million to US$6.6 million[58].

Future directions

There are a number of areas that need to be addressed to improve the standard and quality of research into medicinal mushrooms as an adjuvant cancer treatment. First is the need to conduct further high quality research into specific medicinal mushrooms for individual cancers. This is necessary so that detailed information is gathered on the individual treatment options for specific cancer patients. Second is the requirement that this research be conducted on humans in the form of randomised controlled trials.

Third is the need to standardise the type of extract and dose of the individual medicinal mushrooms used in trials. It is difficult to assess the impact of an intervention if a standardised extract and dose is not used in a trial.

Conclusion

Despite the challenges of conducting research into medicinal mushrooms, further high quality clinical research is justified and needed to investigate the potential role of individual medicinal mushrooms as an adjuvant treatment in breast cancer. The research should focus on the role of specific medicinal mushrooms (in this case, *G. lucidum*) on QoL measures (for example, the cancer patient QoL questionnaire, the EORTC QLC-C30[59]) and immunological measures (such as T-lymphocyte, cytokine and inflammatory biomarkers) in breast cancer treatment. The research could be conducted during or after conventional chemotherapy treatment to assess any benefit from the intervention. A standardised extract and dose of *G. lucidum* would have to be used and a specified breast cancer staging would be required for the research project (for example, Stage II/III) to allow meaningful research results. Clinical research is warranted given the high use of CM by cancer patients, and breast cancer patients in particular, and given the potential benefits in reducing the side effects (such as CRF) associated with conventional cancer treatment. Providing patients with evidence-based medicine recommendations and improving their QoL is a priority when supporting people who are confronted with a diagnosis of breast cancer.

Conflict of interest

The authors declare no conflicts of interest.

Funding

The authors received no funding for this study.

References

1. Wasser SP. Medicinal mushroom science: current perspectives, advances, evidences, and challenges. Biomedical Journal 2014;37(6):345–56.
2. Smith JE, Rowan NJ, Sullivan R. Medicinal mushrooms: a rapidly developing area of biotechnology for cancer therapy and other bioactivities. Biotechnology Letters 2002;24(22):1839–45.
3. Sullivan R, Smith JE, Rowan NJ. Medicinal mushrooms and cancer therapy: translating a traditional practice into Western medicine. Perspectives in Biology and Medicine 2006;49(2):159–70.
4. Figueiredo L, Régis WCB. Medicinal mushrooms in adjuvant cancer therapies: an approach to anticancer effects and presumed mechanisms of action. Nutrire 2017;42(28):1–10.
5. Blagodatski A, Yatsunskaya M, Mikhailova V, Tiasto V, Kagansky A, Katanaev VL. Medicinal mushrooms as an attractive new source of natural compounds for future cancer therapy. Oncotarget 2018;9(49):29259–74.
6. Liu Q, Tie L. Preventive and therapeutic effect of Ganoderma (Lingzhi) on diabetes. Advances in Experimental Medicine and Biology 2019;1182:201–15.
7. Zheng J, Yang B, Yu Y, Chen Q, Huang T, Li D. *Ganoderma lucidum* polysaccharides exert anti-hyperglycemic effect on streptozotocin-induced diabetic rats through affecting β-cells. Combination Chemistry and High Throughput Screening 2012;15(7):542–50.
8. Ito T, Urushima H, Sakaue M, Yukawa S, Honda H, Hirai K, et al. Reduction of adverse effects by a mushroom product, Active Hexose Correlated Compound (AHCC) in patients with advanced cancer during chemotherapy: the significance of the levels of HHV-6 DNA in saliva as a surrogate biomarker during chemotherapy. Nutrition and Cancer 2014;66(3):377–82.
9. Nagashima Y, Yoshino S, Yamamoto S, Maeda N, Azumi T, Komoike Y, et al. Lentinula edodes mycelia extract plus adjuvant chemotherapy for breast cancer patients: results of a randomized study on host quality of life and immune function improvement. Molecular and Clinical Oncology 2017;7(3):359–66.
10. Venturella G, Ferraro V, Cirlincione F, Gargano ML. Medicinal mushrooms: bioactive compounds, use, and clinical trials. Journal of Molecular Science 2021;22(634):1–30.
11. Jeitler M, Michalsen A, Frings D, Hübner M, Fischer M, Koppold-Liebscher DA, et al. Significance of medicinal mushrooms in integrative oncology: a narrative review. Frontiers in Pharmacology 2020;11:1–13.
12. Dai R, Liu M, Nik Nabil WN, Xi Z, Xu H. Mycomedicine: a unique class of natural products with potent anti-tumour bioactivities. Molecules 2021;26(4):1–22.
13. Ebede CC, Jang Y, Escalante CP. Cancer-related fatigue in cancer survivorship. The Medical Clinics of North America 2017;101(6):1085–97.
14. Thong MSY, van Noorden CJF, Steindorf K, Arndt V. Cancer-related fatigue: causes and current treatment options. Current Treatment Options in Oncology 2020;21(17):1–19.
15. Weis J. Cancer-related fatigue: prevalence, assessment and treatment strategies. Expert Review Pharmacoeconomics & Outcomes Research 2011;11(4):441–6.
16. O'Higgins CM, Brady B, O'Connor B, Walsh D, Reilly RB. The pathophysiology of cancer-related fatigue: current controversies. Supportive Care Cancer 2018;26(10):3353–64.
17. De Silva DD, Rapior S, Fons F, Bahkali AH, Hyde KD. Medicinal mushrooms in supportive cancer therapies: an approach to anti-cancer effects and putative mechanisms of action. Fungal Diversity 2012;55(1):1–35.
18. Zhong L, Yan P, Lam WC, Yao L, Bian Z. *Coriolus versicolor* and *Ganoderma lucidum* related natural products as an adjunct therapy for cancers: a systematic review and meta-analysis of randomized controlled trials. Frontiers in Pharmacology 2019;10(703):1–14.
19. Fritz H, Kennedy DA, Ishii M, Fergusson D, Fernandes R, Cooley K, et al. Polysaccharide K and *Coriolus versicolor* extracts for lung cancer: a systematic review. Integrative Cancer Therapies 2015;14(3):201–11.
20. Hong SA, Kim K, Nam S-J, Kong G, Kim MK. A case-control study on the dietary intake of mushrooms and breast cancer risk among Korean women. International Journal of Cancer 2008;122(4):919–23.
21. Shin A, Kim J, Lim SY, Kim G, Sung MK, Lee ES, et al. Dietary mushroom intake and the risk of breast cancer based on hormone receptor status. Nutrition and Cancer 2010;62(4):476–83.
22. Zhang M, Huang J, Xie X, Holman CDAJ. Dietary intakes of mushrooms and green tea combine to reduce the risk of breast cancer in Chinese women. International Journal of Cancer 2009;124(6):1404–8.
23. Ba DM, Ssentongo P, Beelman RB, Muscat J, Gao X, Richie JP, Jr. Higher mushroom consumption is associated with lower risk of cancer: a systematic review and meta-analysis of observational studies. Advances in Nutrition 2021;1–11.
24. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. Cancer Journal for Clinicians 2021;71(3):209–49.
25. Nardin S, Mora E, Varughese FM, D'Avanzo F, Vachanaram AR, Rossi V, et al. Breast cancer survivorship, quality of life, and late toxicities. Frontiers in Oncology 2020;11:1–6.
26. Loprinzi PD, Cardinal BJ. Effects of physical activity on common side effects of breast cancer treatment. Breast Cancer 2012;19(1):4–10.
27. Leggett S, Koczwara B, Miller M. The impact of complementary

and alternative medicines on cancer symptoms, treatment side effects, quality of life, and survival in women with breast cancer: a systematic review. Nutrition and Cancer 2015;67(3):373–91.
28. Nayeri ND, Bakhshi F, Khosravi A, Najafi Z. The effect of complementary and alternative medicines on quality of life in patients with breast cancer: a systematic review. Indian Journal of Palliative Care 2020;26(1):95–104.
29. Unlu A, Nayir E, Kirca O, Ozdogan M. *Ganoderma lucidum* (Reishi mushroom) and cancer. Jnl Balken Union of Oncology 2016;21(4):792–8.
30. Cheng S, Sliva D. *Ganoderma lucidum* for cancer treatment: we are close but still not there. Integrative Cancer Therapies 2015;14(3):249–57.
31. Baby S, Johnson AJ, Govindan B. Secondary metabolites from Ganoderma. Phytochemistry 2015;114:66–101.
32. Hu H, Ahn N-S, Yang X, Lee Y-S, Kang K-S. *Ganoderma lucidum* extract induces cell cycle arrest and apoptosis in MCF-7 human breast cancer cell. International Journal of Cancer 2002;102(3):250–3.
33. Sliva D, Labarrere C, Slivova V, Sedlak M, Lloyd FP, Jr., Ho NW. *Ganoderma lucidum* suppresses motility of highly invasive breast and prostate cancer cells. Biochemical and Biophysical Research Communications 2002;298(4):603–12.
34. Jiang J, Grieb B, Thyagarajan A, Sliva D. Ganoderic acids suppress growth and invasive behavior of breast cancer cells by modulating AP-1 and NF-κB signaling. International Journal of Molecular Medicine 2008;21(5):577–84.
35. Li F, Wang Y, Wang X, Li J, Cui H, Niu M. Ganoderic acids suppress growth and angiogenesis by modulating the NF-κB signaling pathway in breast cancer cells. International Journal of Clinical Pharmacology and Therapeutics 2012;50(10):712–21.
36. Wu GS, Lu JJ, Guo JJ, Li YB, Tan W, Dang YY, et al. Ganoderic acid DM, a natural triterpenoid, induces DNA damage, G1 cell cycle arrest and apoptosis in human breast cancer cells. Fitoterapia 2012;83(2):408–14.
37. Martínez-Montemayor MM, Acevedo RR, Otero-Franqui E, Cubano LA, Dharmawardhane SF. *Ganoderma lucidum* (Reishi) inhibits cancer cell growth and expression of key molecules in inflammatory breast cancer. Nutrition and Cancer 2011;63(7):1085–94.
38. Suarez-Arroyo IJ, Rosario-Acevedo R, Aguilar-Perez A, Clemente PL, Cubano LA, Serrano J, et al. Anti-tumor effects of *Ganoderma lucidum* (Reishi) in inflammatory breast cancer in in vivo and in vitro models. PloS One 2013;8(2):1–12.
39. Zhang Y. *Ganoderma lucidum* (Reishi) suppresses proliferation and migration of breast cancer cells via inhibiting Wnt/β-catenin signaling. Biochemical and Biophysical Research Communications 2017;488(4):679–84.
40. Acevedo-Díaz A, Ortiz-Soto G, Suárez-Arroyo IJ, Zayas-Santiago A, Martínez Montemayor MM. *Ganoderma lucidum* extract reduces the motility of breast cancer cells mediated by the RAC-lamellipodin axis. Nutrients 2019;11(5):1–14.
41. Jiao C, Chen W, Tan X, Liang H, Li J, Yun H, et al. *Ganoderma lucidum* spore oil induces apoptosis of breast cancer cells in vitro and in vivo by activating caspase-3 and caspase-9. Journal of Ethnopharmacology 2020;247:1–9.
42. Loganathan J, Jiang J, Smith A, Jedinak A, Thyagarajan-Sahu A, Sandusky GE, et al. The mushroom *Ganoderma lucidum* suppresses breast-to-lung cancer metastasis through the inhibition of pro-invasive genes. International Journal of Oncology 2014;44(6):2009–15.
43. Rios-Fuller TJ, Ortiz-Soto G, Lacourt-Ventura M, Maldonado-Martinez G, Cubano LA, Schneider RJ, et al. *Ganoderma lucidum* extract (GLE) impairs breast cancer stem cells by targeting the STAT3 pathway. Oncotarget 2018;9(89):35907–21.
44. Su J, Li D, Chen Q, Li M, Su L, Luo T, et al. Anti-breast cancer enhancement of a polysaccharide from spore of *Ganoderma lucidum* with Paclitaxel: suppression on tumor metabolism with gut microbiota reshaping. Frontiers in Microbiology 2018;9:1–14.
45. Roda E, Luca F, Iorio CD, Ratto D, Siciliani S, Ferrari B, et al. Novel medicinal mushroom blend as a promising supplement in integrative oncology: a multi-tiered study using 4T1 triple-negative mouse breast cancer model. International Journal of Molecular Science 2020;21(10):1–28.
46. Deng Y, Ma J, Tang D, Zhang Q. Dynamic biomarkers indicate the immunological benefits provided by Ganoderma spore powder in post-operative breast and lung cancer patients. Clinical and Translational Oncology 2021;23(7):1481–90.
47. Zhao H, Zhang Q, Zhao L, Huang X, Wang J, Kang X. Spore powder of *Ganoderma lucidum* improves cancer-related fatigue in breast cancer patients undergoing endocrine therapy: a pilot clinical trial. Evidence Based Complementary and Alternative Medicine 2012;2012:1–8.
48. Bao P-P, Lu W, Cui Y, Zheng Y, Gu K, Chen Z, et al. Ginseng and *Ganoderma lucidum* use after breast cancer diagnosis and quality of life: a report from the Shanghai breast cancer survival study. PloS One 2012;7(6):1–9.
49. Zeng P, Chen Y, Zhang L, Xing M. *Ganoderma lucidum* polysaccharide used for treating physical frailty in China. Progress in Molecular Biology and Translational Science 2019;163:179–219.
50. Jin X, Ruiz Beguerie J, Sze DMY, Chan GCF. *Ganoderma lucidum* (Reishi mushroom) for cancer treatment. Cochrane Database of Systematic Reviews 2016;4.
51. Corso CR, Mulinari Turin de Oliveira N, Moura Cordeiro L, Sauruk da Silva K, da Silva Soczek SH, Frota Rossato V, et al. Polysaccharides with antitumor effect in breast cancer: a systematic review of non-clinical studies. Nutrients 2021;13(6):1–25.
52. Liu H, Wang ZY, Zhou YC, Song W, Ali U, Sze DM. Immunomodulation of Chinese herbal medicines on NK cell populations for cancer therapy: a systematic review. Journal of Ethnopharmacology 2021;268:1–24.
53. Lam CS, Cheng LP, Zhou LM, Cheung YT, Zuo Z. Herb-drug interactions between the medicinal mushrooms *Lingzhi* and *Yunzhi* and cytotoxic anticancer drugs: a systematic review. Chinese Medicine 2020;15(75):1–18.
54. Rizzo G, Goggi S, Giampieri F, Baroni L. A review of mushrooms in human nutrition and health. Trends in Food Science and Technology 2021.
55. Hsu K-D, Cheng K-C. From nutraceutical to clinical trial: frontiers in Ganoderma development. Applications in Microbial Biotech 2018;102(21):9037–51.
56. Ahmad MF. *Ganoderma lucidum*: a rational pharmacological approach to surmount cancer. Journal of Ethnopharmacology 2020;260:1–12.
57. HealthConsult. Development of a table of standard costs for conducting clinical trials in Australia Sydney; 2013 [cited 2021 Aug 14]. Available from: http://www.healthconsult.com.au/wp-content/uploads/2013/12/CTAG-Determination-final-report.pdf
58. Sertkaya A, Wong HH, Jessup A, Beleche T. Key cost drivers of pharmaceutical clinical trials in the United States. Clinical Trials 2016;13(2):117–26.
59. EORTC Quality of Life Group. EORTC QLG-C30 (Version 3); 1995 [cited 2021 Nov 29]. Available from: https://www.eortc.org/app/uploads/sites/2/2018/08/Specimen-QLQ-C30-English.pdf

Reviews of articles on medicinal herbs

Dr Wendy McLean

These abstracts are brief summaries of articles which have appeared in recent issues of herbal medicine journals, some of which may be held in the NHAA library.

Green tea/green tea extracts and liver biomarkers

Fallah S, Musa-Veloso K, Cao J, Venditti C, Lee HY, Hamamji S, et al. Liver biomarkers in adults: evaluation of associations with reported green tea consumption and use of green tea supplements in US NHANES. Regulatory Toxicology and Pharmacology 2022;129:105087.

There have been isolated case reports of liver toxicity in individuals consuming supplements containing green tea extracts (GTE), questioning the safety of green tea and green tea supplements. It has been reported that their excessive consumption (10–29mg/kg body weight/day) as a supplement, but not green tea consumed as part of a diet or beverage, is associated with oxidative stress-induced hepatotoxicity or acute hepatitis-like syndrome. However, data on the safety of green tea and products containing green tea or GTE at a population level are lacking.

In the current study, the association between consumption of green tea or green tea supplements and abnormal liver biomarkers in adults was investigated using cross-sectional data from the 2009–2014 United States National Health and Nutrition Examination Survey (US NHANES). Dietary survey data were collected from individuals and households via 24-hour dietary recalls administered on two non-consecutive days. Dietary intake data were available for 15,774 individuals aged 19 years or older, and liver biomarkers (i.e. bilirubin, gamma-glutamyl transferase (GGT), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP)) were available for 14,924 of these individuals. Individuals with levels of either bilirubin or GGT, ALT, AST and/or ALP over the age- and gender-specific upper limits of normal ranges were classified as having abnormal liver biomarkers. Steeped green tea consumption was quantified in three ways: as consumption versus no consumption, servings/doses per day, and amount (g) per day. Use of green tea supplements (containing green tea or a GTE) was quantified as consumption versus no consumption and servings/doses per day. The associations between green tea and green tea supplement use and the odds of having abnormal liver function were assessed using multiple logistic regression models that included other confounding variables (i.e. gender, age, race, medication use, alcohol use, waist circumference or liver disease).

In total, 12,289 persons were included in the green tea analyses and 12,274 in the green tea supplement analyses. Twenty-three percent of individuals included in the analysis had at least one abnormal liver biomarker. Among the total population, the percentage of consumers of green tea was 2.10%, with a mean intake frequency of approximately 1.2 servings/doses per day and a mean intake of 620g/day. The odds of having one or more abnormal liver biomarkers were reduced by nearly half with the consumption of green tea (OR=0.48). However, when the multivariate regression analysis was limited to green tea users, there was no relationship between the amount of green tea consumed or the number of green tea consumption occasions and the odds of having an abnormal liver function. The percentage of users of green tea supplements was 1.43%. Consumption of green tea supplements versus no consumption was not associated with abnormal liver markers.

In summary, the results indicate consuming steeped green tea is associated with a significant reduction in the odds of having one or more abnormal liver biomarkers, suggesting a possible protective effect of green tea consumption on liver function. However, it is important to note that the NHANES study is a cross-sectional study, so observations are associations only, and no conclusions can be made on cause-and-effect relationships. Furthermore, the data were insufficient to quantify the amount/strength of green tea or catechin composition of GTE or determine which liver biomarkers are more affected by consumption of green tea and/or green tea supplements. More research is required to determine whether GTE-related hepatoxic effects are dose-dependent or attributable to the presence of other ingredients in the supplements.

Curcumin and Boswellia in IBS and small bowel dysbiosis

Giacosa A, Riva A, Petrangolini G, Allegrini P, Fazia T, Bernardinelli L, et al. Beneficial effects on abdominal bloating with an innovative food-grade formulation of Curcuma longa and Boswellia serrata extracts in subjects with irritable bowel syndrome and small bowel dysbiosis. Nutrients 2022 Jan;14(3):416.

Abdominal bloating is one of the most common symptoms reported by individuals with irritable bowel syndrome (IBS) and is a significant cause of psychological distress and reduced quality of life. IBS is characterised by gut microbial dysbiosis, and gut microbiota could play a key role in the pathogenesis of this syndrome. Small intestinal bacterial overgrowth (SIBO), an intestinal microbiome dysbiosis, appears to be commonly associated with bloating and IBS.

Growing evidence supports the efficacy of microbiota-directed therapies, including prebiotics, probiotics and dietary interventions such as low FODMAP (Fermentable Oligosaccharides, Disaccharides, Monosaccharides and Polyols) diets in the treatment of IBS. In addition, herbal extracts with anti-inflammatory and gut antimicrobial effects, such as *Curcuma longa* and *Boswellia serrata*, may be useful in managing both IBS and SIBO. Therefore, this study aimed to compare the effectiveness of curcumin and boswellia extracts (as Curcumin Boswellia Phytosome (CBP)) plus a low FODMAP diet (LFD) compared to a low FODMAP diet alone on the relief of abdominal bloating in IBS subjects with small bowel dysbiosis.

The study was a randomised controlled trial including 67 participants, aged 18–70 years, diagnosed with IBS (Rome III criteria) and small bowel dysbiosis who experienced bloating. Small bowel dysbiosis was defined by increased urinary indican with normal urinary skatole. Exclusion criteria included subjects already on LFD or other dietary restriction, normal urinary indican values, or a history of inflammatory bowel, renal or liver disease. Patients were randomised to either the curcumin and boswellia extracts (500mg twice daily plus LFD) (n=33) or control arm (LFD alone) (n=34) for 30 days. Abdominal bloating and pain scores and intestinal dysbiosis (urinary indican and skatole) were assessed at baseline and at 30 days. Patients completed a global assessment of efficacy at the end of the study.

Baseline characteristics were similar between groups. The intervention group (33 participants) showed a significant decrease in bloating, abdominal pain and indican values at 30 days compared to the control group. Furthermore, at the end of the study, 33 participants of the intervention group reported no or a mild degree of bloating compared to only 10 subjects in the control group. The intervention group showed a significantly better global assessment of efficacy (GAE) than the control group.

In conclusion, supplementation with CBP plus LFD effectively improved abdominal bloating in subjects with IBS and small bowel dysbiosis compared to LFD alone. However, there are several limitations to the study, including the short duration and the results need to be confirmed by trials of a longer duration of supplementation and studies that investigate the clinical mechanisms of effect of combined *Curcuma longa* and *Boswellia serrata* against placebo controls in people with bloating due to IBS.

Ashwagandha for perimenopause

Gopal S, Ajgaonkar A, Kanchi P, Kaundinya A, Thakare V, Chauhan S, et al. Effect of an ashwagandha (Withania Somnifera) root extract on climacteric symptoms in women during perimenopause: a randomized, double-blind, placebo-controlled study. Journal of Obstetrics and Gynaecology Research 2021;47(12):4414–4425.

During perimenopause, women experience various symptoms that can significantly impair their quality of life, including physical and psychological health. For preventing and managing these various climacteric symptoms, hormone replacement therapy (HRT) is frequently used; however, it is occasionally associated with an increased risk of blood clots and breast cancer. Therefore, alternative treatments, such as herbal medicines, are gaining popularity.

Withania somnifera (known as ashwagandha or withania) is a medicinal plant with a long history of use in Ayurvedic medicine to treat stress and anxiety. Ashwagandha has anti-inflammatory, neuroprotective and cognitive-enhancing effects. It has also been found to influence sex hormones and has been used to treat infertility and sexual dysfunction in men and women. Due to the promising findings of ashwagandha administration on reproductive health in women, this study aimed to examine the efficacy and tolerability of an ashwagandha root extract on the climacteric symptoms, quality of life (QoL), and hormonal parameters in perimenopausal women.

This 8-week, randomised, double-blind, placebo-controlled study included 100 healthy perimenopausal women aged 45–60 years. Participants had a BMI between 18–35kg/m^2, at least two missing periods during the past 12 months, and suffered perimenopausal symptoms. Exclusion criteria included herbal extract or HRT use, present active medical, surgical and gynaecological problems, history of drug or alcohol abuse, history of breast or cervical carcinoma or mental/cognitive condition. Participants were randomly allocated to take either a placebo or 300mg of an ashwagandha root extract twice daily. Outcomes were measured using the menopause rating scale (MRS), menopause-specific QoL (MENQoL), hot flash score, and hormonal changes in oestradiol, follicle-stimulating hormone (FSH), luteinising hormone (LH) and testosterone. A trained clinician evaluated participants at baseline, week 4 and week 8.

Ninety-one participants completed the study comprising 46 participants in the ashwagandha group and 45 participants in the placebo group. At the end of the 8 weeks, women in the ashwagandha reported a statistically significant reduction in total MRS score compared to women taking placebo controls, reflected by significant reductions in the psychological, somato-vegetative and urogenital domains. There was a statistically significant reduction in the total MENQoL score in the ashwagandha group compared to the placebo group. Women supplemented with ashwagandha experienced a significant reduction in their mean daily number of hot flashes and sweating. Ashwagandha intake was also found to significantly increase in serum oestradiol and significantly reduce serum FSH and serum LH compared with the placebo. There was no significant between the group differences in the serum testosterone level. Ashwagandha intake was well-tolerated, with no major adverse events reported. The frequency of mild adverse events was slightly higher in the placebo group, which could be attributed to the menopausal transition itself.

Although the findings from this study provide preliminary evidence for the beneficial effects of ashwagandha for alleviating climacteric symptoms, there are several limitations to the study, including the small sample size and short study duration. Further research is required to understand the potential role of ashwagandha in preventing and managing climacteric symptoms in perimenopausal women.

Black cumin oil for sleep disturbances

Syam Das S, Kannan R, George S, Chakrapani B, Maliakel B, Ittiyavirah S, Krishnakumar IM. Thymoquinone-rich black cumin oil improves sleep quality, alleviates anxiety/stress on healthy subjects with sleep disturbances: a pilot polysomnography study. Journal of Herbal Medicine 2022;32:100507.

Inadequate sleep is a universal health problem, affecting more than one in four people. Depression, anxiety and stress can have a significant role in the development of insomnia which is the most common sleep disorder. Herbal medicines have a long history of use for nervous system disorders and, as such, there is growing interest in these medicines for improving sleep quality. However, most of these natural sleep aids need a heavy dosage (>600mg/day) to produce a significant effect, and their efficacy is often debated.

Nigella sativa (NS), commonly known as black cumin or black seed, is a widely used medicinal herb. Most of the therapeutic properties of this plant are dedicated to the seed oil in which thymoquinone (TQ). Experimental studies with NS extracts report anti-anxiety, antidepressant and sleep improving effects. Therefore, the present pilot study investigated the efficacy of the safety and effectiveness of a thymoquinone-rich (5% w/w) black cumin oil (BCO) on healthy subjects having significant stress, anxiety and sleep disturbances.

The study included 18 subjects aged 35–65 years who experienced at least one sleep complaint, such as difficulty initiating sleep, which was present for 3 or more nights per week for at least 3 months. Exclusion criteria included having an allergy to black cumin, low blood pressure or a history of depression or other mental disorder. Participants consumed one soft gel capsule of BCO (200mg) after dinner for 28 days. The primary objectives were the sleep parameters such as the change in sleep latency, sleep duration, wake-after-sleep-onset (WASO) time, and sleep efficiency. These were assessed using polysomnographic analysis performed at the beginning and after 7 days of supplementation. In addition, sleep quality index, anxiety and stress (secondary outcomes) were also measured at the beginning and the end of the study period using the validated questionnaires, Pittsburgh Sleep Quality Index (PSQI), Depression Anxiety Stress Scale-21 (DASS-21) and Hamilton Anxiety Rating Scale-A (HAM-A), along with the measurements of cortisol and safety parameters.

Fifteen participants completed the study. Polysomnography analysis on day 7 showed significant improvements in primary outcomes such as total sleep time, sleep latency and sleep efficiency with an increase of 82.49% in non-rapid eye movement (NREM 3) and 29.38% increase in rapid eye movement sleep (REM sleep). There was also a significant reduction in PSQI score on day 28 (39.72%), which indicates improvement in sleep quality and correlating with the polysomnography results. There was a significant reduction in DASS-21 scores; however, among the three sub-dimensions of DASS-21 (anxiety, stress and depression), only the stress and anxiety scores showed a significant decrease at the end of the study period compared to baseline scores. The observed reduction in DASS-21 was also supported by the results of HAM-A, which showed a 23.94% reduction in anxiety. In addition, the cortisol level showed a significant reduction at the end of the study (29.72%) compared to the baseline values. There were no significant changes in haematological or biochemical parameters, demonstrating the safety of BCO during the study period.

Strengths of the study include using polysomnographic analysis and correlating the results with questionnaires and blood cortisol levels. Significant limitations include the small sample size and lack of a control group. Nevertheless, the study results suggest a positive effect of BCO for improving sleep quality and ameliorating stress and anxiety. Further randomised-controlled studies are warranted.

Probiotic and herbal combination for acne

Rinaldi F, Marotta L, Mascolo A, Amoruso A, Pane M, Giuliani G, et al. Facial acne: a randomized, double-blind, placebo-controlled study on the clinical efficacy of a symbiotic dietary supplement. Dermatology and Therapy 2022;1–13.

Acne vulgaris (acne) is a common yet complex inflammatory skin condition. The proliferation of bacterium, including *Cutibacterum acnes*, *Staphylococcus aureus* and *Staphylococcus epidermis*, plays a crucial role in eliciting a host inflammatory response that is thought to be important for the pathogenesis of the disease. Standard therapies for acne, such as topical and oral antibiotics and retinoids, have many limitations, including skin and gut microbiome disruption. Therefore, alternative treatments are needed to restore the dysbiosis correlated with acne onset and evolution.

There is growing evidence supporting the use of probiotics for acne treatment, with their beneficial effects attributed to their anti-inflammatory properties, ability to maintain skin hydration and barrier function, and correct microbiome dysbiosis. More recently, there has been interest in botanical extracts and plant-derived secondary metabolites for managing inflammatory skin conditions such as acne. Lupeol, a pentacyclic triterpene from *Solanum melongena*, is one plant metabolite that has been reported to target the main pathogenic features of acne. Another natural alternative is Echinacea, which has antimicrobial and anti-inflammatory effects and demonstrated efficacy for other skin lesions, including wound healing. Therefore, the purpose of the current study was to test the effectiveness of a dietary supplement

containing probiotics and a botanical extract in subjects with mild to moderate acne over an 8-week study period. The probiotics included *Bifidobacterium breve* BR03 DSM 16604, *Lacticaseibacillus casei* LC03 DSM 27537, and *Ligilactobacillus salivarius* LS03 DSM 22776 and the botanical extract contained lupeol from *Solanum melongena L.* and Echinacea extract.

The study was a randomised, double-blind, four-arm, placebo-controlled clinical study including adult subjects (average age 23 years) with mild to moderate acne, recruited at a dermatologic clinic in Italy. Exclusion criteria included current use of any prescription treatment (oral or topical) for acne (2 months washout allowed), pregnancy and lactation, or known allergy or hypersensitivity to any of the constituents of the study product. Subjects were randomised to one of four groups: (I) placebo, (II) probiotic + botanical extract, (III) botanical extract and (IV) probiotics. Outcomes included changes from baseline in the number of lesions, erythema, desquamation, sebum level and microbial dysbiosis. These were assessed at baseline and 4 and 8 weeks after treatment.

In total, 114 of the enrolled subjects (95%) completed the study (28 in group I, 30 in group II, 29 in group III, and 27 in group IV). A significant effect on the number of superficial inflammatory lesions was reported over the study period in the subjects taking the probiotic + botanical extract (group II) (–57%), the botanical extracts (group III) (–40%), and the probiotics (group IV) (–39%) versus placebo (–10%). Other statistically significant outcomes at 8 weeks included decreased mean desquamation score, sebum secretion rate, and porphyrin mean count in the three treatment groups. In addition, compared with baseline, after 4 and 8 weeks of treatment, there was a significant decrease in *C. acnes* and an increase in *S. epidermis* in the three treatment groups. Also, *S. aureus* was significantly reduced in groups II and III at 4 and 8 weeks. Overall, the most significant before and after effects were observed in group II (probiotic + botanical extract).

Some limitations apply to the study, including its short-duration intervention, single site, relatively small population, and the possibility that non-inflammatory lesions may resolve independently. However, the results suggest that a dietary supplement containing probiotics and a botanical extract is a promising and safe treatment for inflammatory acne. Larger randomised, controlled trials are needed to substantiate the clinical effects on person-centred outcomes of importance to people with acne.

Silymarin and cardiometabolic syndrome

Soleymani S, Ayati MH, Mansourzadeh MJ, Namazi N, Zargaran, A. The effects of Silymarin on the features of cardiometabolic syndrome in adults: a systematic review and meta-analysis. Phytotherapy Research 2022;10.1002/ptr.7364. Advance online publication.

Cardiometabolic syndrome (CMS) is a significant public health concern, affecting 25% of the global population. This syndrome involves clustering of metabolic and cardiovascular factors that increase the risk of patients developing Type 2 Diabetes Mellitus (TD2M) and cardio/cerebrovascular disease. Management of CMS primarily involves pharmaceutical interventions; however, these can be associated with side effects leading to poor patient compliance. Accordingly, researchers have focused on complementary therapies to manage such cardiometabolic dysfunctions, such as herbal medicines.

The primary active constituents in *Silybum marianum* (St Mary's Thistle) are the flavonolignans, collectively known as silymarin. Pharmacological studies have demonstrated silymarin's hepatoprotective, antioxidant, anti-inflammatory and cardioprotective effects. In addition, previous meta-analyses demonstrate the beneficial effects of silymarin on metabolic status; however, these only focused on patients with T2DM. Therefore, the present systematic review and meta-analysis aimed to examine the effects of silymarin on the components of CMS in adults.

Four electronic databases, PubMed/MEDLINE, Scopus, Web of Science and Embase, were systematically searched up to 31 December 2020 to identify all eligible clinical trials. Randomised controlled trials were eligible for inclusion if they compared silymarin with placebo or a control group and provided sufficient data at baseline and the end of the study for the features of CMS (such as anthropometric indices, glycaemic status, lipid profile, and blood pressure). Studies that included children or silymarin as an adjunct therapy were not eligible for inclusion. A random-effect model was used to pool effect sizes for all primary and secondary outcomes. The Jadad checklist was used to assess the quality of the included clinical trials.

The search identified 11 eligible studies, providing outcome data for 816 subjects aged 18–73 years. Daily dosages of silymarin ranged from 140–2,100mg/day with a follow-up period between 45 days and 12 months. The effects of silymarin were examined in patients with T2DM (n=6), type 1 diabetes mellitus (n=1), non-alcoholic steatohepatitis, and non-alcoholic fatty liver disease (NAFLD) (n=2). Based on the Jadad score, most clinical trials (n=8) had high methodological quality (score ≥3), and the quality of the rest was low (score <3).

Meta-analysis showed that supplementation with silymarin significantly decreased serum levels of fasting blood sugar (FBS), glycated haemoglobin (HbA1c), total cholesterol, triglycerides, and low-density lipoprotein (LDL) cholesterol compared to placebo. In participants who consumed silymarin, serum levels of high-density lipoprotein (HDL)-C were increased significantly compared to those in the placebo group. Silymarin supplementation did not reduce BMI compared to placebo. Subgroup analysis indicated that the effects of silymarin on the features of CMS in patients with

diabetes were greater than non-diabetic ones. In addition, subgroup analysis showed that silymarin with dosages of more than 420mg/day was more effective than lower ones, and its effects were greater in older patients (>50 years). All the included clinical studies except two did not report any adverse effects for supplementation with silymarin.

The results suggest that silymarin may be a safe and effective complementary therapy for improving the main components of CMS, particularly in patients with diabetes. However, significant heterogeneity was identified in trials which limits the strength of findings, and further high-quality trials are required to confirm silymarin's efficacy for CMS.

Vitex agnus-castus and *Salvia officinalis* extracts on serum lipids in postmenopausal women

Zeidabadi A, Jafari M, Emamghoreishi M, Sasani MR, Akbarzadeh M. Effect of Vitex agnus-castus and Salvia officinalis extracts on serum lipids in postmenopausal women: a randomized clinical trial. International Journal of Women's Health and Reproduction 10(1).

The menopause transition is characterised by changes in hormones and body composition that increase overall cardiometabolic risk. Hormone replacement therapy is often used to reduce cardiometabolic risk. However, considering the side effects of hormone replacement therapy, many women seek alternative treatments such as herbal medicines.

Complementary medicines commonly used to alleviate menopausal symptoms include Vitex agnus-castus (VAC) (chaste tree berry) and *Saliva officinalis* (sage). Both herbs contain bioactive constituents with phytoestrogenic properties. Phytoestrogens have antioxidant and anti-inflammatory effects and studies have shown their potential to improve cardiovascular function and decrease the risk of cardiovascular disease (CVD) associated with menopause. Therefore, the current study aimed to investigate the effect of VAC and *S. officinalis* extracts on serum lipids in postmenopausal women.

This 3-month randomised clinical trial included 99 postmenopausal women referred to a bone densitometry centre in Iran. Women who had been menopausal for at least 1 year and not taking steroid hormones, hormonal treatments, herbal medicines or drugs affecting serum lipids were eligible for inclusion. Initially, 99 women were randomised to one of three groups, VAC (n=33), *S. officinalis* (n=33) or placebo (n=33). The VAC tablets contained 3.2–4.8mg of dried extract of VAC fruits standardised to contain 0.42–0.55mg Aucubin (an iridoid glycoside). Each *S. officinalis* tablet contained 100mg of a dried extract standardised to contain 19–25mg tannins (acid tannic). The primary outcome was the change in the total serum cholesterol, low-density lipoprotein (LDL), triglycerides (TG), and high-density lipoprotein (HDL) after the 3-month intervention.

A total of 89 participants completed the study, with ten excluded due to poor compliance. The average age of participants was 56 years. Baseline demographics, body mass index (BMI) and menopausal period were similar between groups. Compared to the baseline, a significant decrease in serum total cholesterol, low-density lipoprotein, and triglycerides levels and also an increase in mean serum high-density lipoprotein levels were observed in VAC and *S. officinalis* groups. In comparison, no significant change was observed in the serum level of any lipoproteins in the placebo group.

The authors reported the results to demonstrate that VAC and *S. officinalis* effectively lower blood lipid levels in postmenopausal women; however, noting limitations. The small sample analyses and longer duration studies (6–12 months) are required to understand the long-term impact of these herbs on cardiometabolic risks in menopausal women.

References

Fallah S, Musa-Veloso K, Cao J, Venditti C, Lee HY, Hamamji S, et al. Liver biomarkers in adults: evaluation of associations with reported green tea consumption and use of green tea supplements in US NHANES. Regulatory Toxicology and Pharmacology 2022;129:105087.

Giacosa A, Riva A, Petrangolini G, Allegrini P, Fazia T, Bernardinelli L, et al. Beneficial effects on abdominal bloating with an innovative food-grade formulation of Curcuma longa and Boswellia serrata extracts in subjects with irritable bowel syndrome and small bowel dysbiosis. Nutrients 2022 Jan;14(3):416.

Gopal S, Ajgaonkar A, Kanchi P, Kaundinya A, Thakare V, Chauhan S, et al. Effect of an ashwagandha (Withania Somnifera) root extract on climacteric symptoms in women during perimenopause: a randomized, double-blind, placebo-controlled study. Journal of Obstetrics and Gynaecology Research 2021;47(12):4414–4425.

Syam Das S, Kannan R, George S, Chakrapani B, Maliakel B, Ittiyavirah S, Krishnakumar IM. Thymoquinone-rich black cumin oil improves sleep quality, alleviates anxiety/stress on healthy subjects with sleep disturbances: a pilot polysomnography study. Journal of Herbal Medicine 2022;32:100507.

Rinaldi F, Marotta L, Mascolo A, Amoruso A, Pane M, Giuliani G, et al. Facial acne: a randomized, double-blind, placebo-controlled study on the clinical efficacy of a symbiotic dietary supplement. Dermatology and Therapy 2022;1–13.

Soleymani S, Ayati MH, Mansourzadeh MJ, Namazi N, Zargaran, A. The effects of Silymarin on the features of cardiometabolic syndrome in adults: a systematic review and meta-analysis. Phytotherapy Research 2022;10.1002/ptr.7364. Advance online publication.

Zeidabadi A, Jafari M, Emamghoreishi M, Sasani MR, Akbarzadeh M. Effect of Vitex agnus-castus and Salvia officinalis extracts on serum lipids in postmenopausal women: a randomized clinical trial. International Journal of Women's Health and Reproduction 10(1).

Reviews of health journal articles

Dr Wendy McLean

These abstracts are brief summaries of articles in recent issues of medical journals. Articles selected are of a general nature for the information of practitioners of naturopathy and herbal medicine. A dominant theme is often present throughout the journals which will be reflected in the reviews.

Olive oil consumption and mortality risk

Guasch-Ferré M, Li Y, Willett WC, Sun Q, Sampson L, Salas-Salvadó J, et al. Consumption of olive oil and risk of total and cause-specific mortality among US adults. Journal of the American College of Cardiology 2022;79(2):101–112.

Olive oil consumption has been associated with a lower risk of cardiovascular disease (CVD). Its benefits for cardiovascular health are due to its anti-inflammatory, antioxidant and antiatherogenic effects. Furthermore, olive oil can improve lipid profile, insulin sensitivity, glycaemic control and endothelial function and decrease blood pressure. However, the association between olive oil consumption and risk of total and cause-specific mortality are unclear. Accordingly, the present study aimed to evaluate the association between total consumption of olive oil and total and cause-specific mortality in two large cohorts of US men and women.

The study analysed olive oil intake from two prospective cohorts of healthy US adults: the Nurses' Health Study (NHS) (1990–2018; n=60,582 women) and the Health Professionals Follow-up Study (HPFS) (1990–2018; n=31,801 men). Participants with a history of CVD or cancer at baseline were excluded. During 28 years of follow-up, diet was assessed using a semi-quantitative food frequency questionnaire (FFQ) every 4 years. Olive oil consumption was categorised by frequency: never or less than once per month (reference); up to 4.5g per day (<1 teaspoon); 4.5–7g per day (1 teaspoon to 0.5 tablespoons); and at least 7g per day (0.5 tablespoons). Multivariable Cox proportional-hazards models were used to estimate hazard ratios (HRs) for total and cause-specific mortality. In addition, statistical substitution models were used to calculate the risk of total and cause-specific mortality when replacing 10g per day of other types of fats (margarine, butter, mayonnaise, other vegetable oils combined [corn, safflower, soybean and canola] and dairy fat) with olive oil.

During 28 years of follow-up, 38,856 participants died (22,768 in NHS and 14,076 in HPFS). Mean olive oil consumption increased from 1.6g per day in 1990 to about 4g per day in 2010. Mean margarine consumption fell from 12g per day in 1990 to 4g per day in 2010. The mean consumption of total olive oil in the highest category (>0.5 tablespoon per day) was 9g per day at baseline and included 5% of the cohort participants.

Compared with participants who rarely or never consumed olive oil, the adjusted pooled HR for all-cause mortality among participants reporting the highest consumption of olive oil was 0.81, with results persisting after adjustments for demographic and lifestyle factors. Furthermore, compared to those with the lowest olive oil intake, those with the highest intake had a 19% lower risk of cardiovascular mortality, 17% lower risk of cancer mortality, 29% lower risk of neurodegenerative mortality and 18% lower risk of respiratory mortality. In substitution analyses, replacing 10g per day of margarine, butter, mayonnaise and dairy fat with the equivalent amount of olive oil was associated with a 13–19% lower risk of total mortality and 8%–34% lower risk of death from CVD, cancer, neurodegenerative and respiratory diseases. No significant associations were observed when olive oil replaced other vegetable oils combined (corn, safflower, soybean and canola oil).

Strengths of the study include the large population size, long-term and high rates of follow-up, and detailed and repeated measurements of diet and lifestyle. Limitations include the possibility of residual confounders and measurement errors associated with self-reported FFQs. Despite these limitations, the findings support current dietary recommendations to increase the intake of olive oil and other unsaturated vegetable oils in place of other fats to lower the risk of chronic disease.

Probiotics improve symptoms and viral clearance in COVID-19

Gutiérrez-Castrellón P, Gandara-Martí T, Abreu Y Abreu AT, Nieto-Rufino CD, López-Orduña E, Jiménez-Escobar I, et al. Probiotic improves symptomatic and viral clearance in Covid-19 outpatients: a randomized, quadruple-blinded, placebo-controlled trial. Gut Microbes 2022;14(1):2018899.

Emerging evidence demonstrates a link between the severity of Coronavirus Disease 2019 (Covid-19) symptoms, mortality and gut microbial dysbiosis, suggesting that targeted manipulation to promote microbial diversity could be an important strategy to treat Covid-19 and enhance recovery. The current study aimed to assess the efficacy and safety of a probiotic formula in symptomatic Covid-19 patients.

The study was a single-centre, quadruple-blinded, randomised trial involving 300 symptomatic Covid-19 patients (18–60 years old) with SARS-CoV-2 confirmation by reverse transcription quantitative real-time PCR (RT-qPCR). Patients with peripheral oxygen saturation (SpO_2) ≥90% and the onset of at least one symptom within ≤7 days of study entry were eligible for

inclusion. Symptoms included fever (>37.5°C), cough, headache, body aches and shortness of breath. Exclusion criteria included uncontrolled diabetes or hypertension, currently immunosuppressed, severe respiratory disease, Body Mass Index (BMI) ≥40kg/m^2, pregnancy or lactation and probiotic or antibiotic use within 2 days before entering the study.

Patients were randomly assigned to probiotic formula (strains *Lactiplantibacillus plantarum* KABP022, KABP023 and KAPB033, plus *Pediococcus acidilactici* KABP021, totalling 2×10^9 colony-forming units (CFU)) or placebo, for 30 days. Endpoints included: i) proportion of patients in complete symptomatic and viral remission; ii) proportion progressing to moderate or severe disease with hospitalisation or death; and iii) days in intensive care unit (ICU). Nasopharyngeal and venous samples, chest pulmonary x-rays and Covid-19 severity were assessed at day 0 (visit 1), day 15 (visit 2) and day 30 (visit 3). In addition, faecal samples were collected at days 0 and 30 for microbiome analysis using 16S rRNA sequencing. Acetaminophen (500mg/dose, up to three times per day) was the only medication allowed for Covid-19 symptoms.

Of the 300 patients randomised, 293 completed the study. The median time from the first symptom to study inclusion was 4 days. Baseline characteristics were generally comparable; however, the probiotic group had a higher incidence of lung infiltrates and lower SpO_2. The probiotic formula achieved a significant effect on improving remission rate on day 30 (i.e. complete symptomatic and viral clearance) against placebo, with complete remission achieved by 78 (53.1%) in the probiotic group compared to 41 (28.1%) in placebo. No hospitalisations, ICU admissions or deaths occurred during the study, preventing the assessment of all pre-specified outcomes.

Probiotic supplementation was well-tolerated and reduced nasopharyngeal viral load and lung infiltrates at days 15 and 30, and duration of both digestive and non-digestive symptoms, compared to placebo. Median time to overall symptom resolution (symptomatic clearance) was 5 days shorter in the probiotic group than in the placebo group. Compared to placebo, probiotic treatment was also associated with higher serum titres of SARS-CoV-2-binding IgG and IgM on days 15 and 30 and lower serum levels of high-sensitivity C-reactive protein (hs-CRP) and D-Dimer on day 15. No significant compositional changes were detected in faecal microbiota between probiotic and placebo groups.

The authors hypothesised that the results demonstrate that this probiotic primarily acts by interacting with the host's immune system rather than changing colonic microbiota composition. However, they note several limitations to the study, including the age (<60 years), single ethnicity (Hispanic) of participants, and lack of assessment of dietary habits, which may influence microbiota composition. Furthermore, the effect on hospitalisation, ICU stay, and mortality could not be assessed because of the lack of occurrences during the study. Future studies are required to replicate these findings in the elderly and other ethnicities and elucidate the mechanism of action.

Depression and cardiometabolic disease

Honigberg MC, Ye Y, Dattilo L, et al. Low depression frequency is associated with decreased risk of cardiometabolic disease. Nature Cardiovascular Research volume 1, pages 125–131 (2022).

Cardiovascular disease (CVD) and depression are global public health concerns and are now recognised as leading causes of death and disability. Epidemiological studies have repeatedly confirmed the high co-morbidity between CVD and depression over the past 40 years. However, it is unclear whether depression contributes to the development of heart disease or whether it is mainly secondary to the clinical condition.

Polygenic risk scores (PRS) are predictive of cardiometabolic disease. These scores are a single value estimate of an individual's genetic liability to a trait or disease, calculated according to their genotype profile and relevant genome-wide association study (GWAS) data. PRS may be useful to identify individuals most likely to benefit from lifestyle and dietary modification. The current study aimed to assess whether the frequency of depressed mood further stratifies polygenic risk of cardiometabolic conditions, independent of lifestyle and conventional CVD risk factors.

Researchers studied the genomes of 328,152 individuals of European ancestry (40–69 years old) available in the UK Biobank between 2006 and 2010. At enrolment, participants provided detailed information on medical history, medication use, lifestyle factors and mental health and underwent physical assessment and blood sample collection for genotyping and pathology analysis, including total and HDL cholesterol and high-sensitivity C-reactive protein (hs-CRP). Participants were followed for the development of incident diagnoses through linkage to national health records and follow-up study visits. The primary study exposure was the self-reported frequency of depressed mood in the previous 2 weeks, ascertained at study enrolment. Researchers assessed the association between depression and PRS for coronary artery disease (CAD), type 2 diabetes (T2D) and atrial fibrillation.

The mean age of participants was 56.8 years, and 53% were female. Overall, 255,078 individuals (77.7%) reported no episodes of depressed mood in the past 2 weeks. Greater frequency of depressed mood was modestly associated with higher CAD PRS and higher T2D PRS but not with atrial fibrillation PRS. However, after adjustment for clinical/lifestyle factors associated with depression and PRS, low versus high frequency of depressed mood was associated with lower risks of incident CAD by 34%, T2D by 33% and atrial fibrillation by 20%. In addition, the association between depression and coronary artery disease was higher in women than in men.

This study expands on current knowledge of the potential contributing role of depression in the development of cardiovascular disease. However, the study results should be interpreted in the context of the study design. Firstly, the primary exposure was assessed using a 2-week recall measure, which can introduce bias. Secondly, the frequency of depressed mood and lifestyle behaviours were assessed only at baseline, and the cumulative and temporal burden of depression, lifestyle risk and other risk factors were not assessed. Further research is needed to clarify mechanisms and implications for preventive care.

Anti-inflammatory diets and rheumatoid arthritis pain – a meta-analysis

Schönenberger KA, Schüpfer AC, Gloy VL, Hasle, P, Stanga Z, Kaegi-Braun N, Reber E. Effect of anti-inflammatory diets on pain in rheumatoid arthritis: a systematic review and meta-analysis. Nutrients 2021;13(12):4221.

Rheumatoid arthritis (RA) is the most common chronic inflammatory rheumatic disease and a significant cause of disability. The pharmacologic therapy consists of disease-modifying anti-rheumatic drugs (DMARDs) and anti-inflammatory treatment with nonsteroidal anti-inflammatory drugs (NSAIDs) or glucocorticoids. However, with known side effects of most of these drugs, interest remains for safe, effective and tolerable anti-inflammatory therapies.

Nutritional therapy for RA aims to attenuate inflammation by altering the ratio of omega-6 to omega-3 fatty acids and increasing antioxidants. Various nutritional therapies have been proposed, particularly diets rich in omega-3 fatty acids, leading to eicosanoid reduction. The authors of the present paper conducted a systematic review and meta-analysis to establish the effect of potentially anti-inflammatory diets (Mediterranean, vegetarian, vegan, ketogenic) on pain in RA.

The authors searched MEDLINE (OVID), Embase (Elsevier) and CINAHL databases for studies published from database inception to 12 November 2021. Studies comparing the effect of a Mediterranean, vegetarian, vegan, or ketogenic diet versus an ordinary omnivorous diet on pain in adults with RA were eligible. Randomised and non-randomised, controlled and uncontrolled trials and observational studies were included. Studies of non-whole diet interventions were excluded, i.e, single food items, nutrients, or supplements. The primary outcome was pain on a 10cm visual analogue scale (VAS). Secondary outcomes were C-reactive protein (CRP) levels, erythrocyte sedimentation rate (ESR), health assessment questionnaire (HAQ), disease activity score 28, tender/swollen joint counts (SJC), weight and body mass index. Meta-analysis of randomised trials was undertaken using random effect models. The risk of bias was assessed using the Cochrane Risk of Bias Tool.

Of 28 studies assessed for eligibility, 12 studies were included in the systematic review and seven in the meta-analysis (n=326). The interventions included the Mediterranean diet (n=5), vegetarian diet (n=4), and vegan diet (n=3). No eligible studies investigated the effect of a ketogenic diet. Study duration ranged from 2 weeks to 13 months. The studies included mainly female patients (92%). All studies had a high risk of bias, and the evidence was very low.

The pooled results showed that overall, patients on anti-inflammatory diets reported significantly less pain than patients in the control groups, improved HAQ and lower SJC. In addition, patients on anti-inflammatory diets lost more weight than patients in the control groups, and BMI decreased. There were no significant differences in CRP, ESR and TJC. Subgroup analysis showed that Mediterranean diets tended to have a more significant effect on pain than vegetarian or vegan diets did. Studies with a longer intervention period (>3 months) tended to have greater effects.

The main limitation of this study is the high risk of bias for the primary outcome. Due to the nature of the research question, it is not possible to blind dietary interventions, and pain measured by VAS is a subjective self-reported outcome. In conclusion, vegetarian, vegan, and Mediterranean diets might benefit some RA patients.

Glycine and N-Acetylcysteine supplementation for type 2 diabetes

Sekhar RV. GlyNAC (Glycine and N-Acetylcysteine) supplementation improves impaired mitochondrial fuel oxidation and lowers insulin resistance in patients with type 2 diabetes: results of a pilot study. Antioxidants 2022;11(1):154.

Type 2 diabetes (T2D) is associated with mitochondrial dysfunction, which involves impaired fatty acid (FA) oxidation, increased reactive oxygen species (ROS) formation, and insulin resistance (IR). Therefore, adequate antioxidant glutathione (GSH) levels are required for optimal mitochondrial fatty-acid oxidation (MFO) and reduced mitochondrial injury or irreversible cell damage due to oxidative stress.

Patients with T2D are reported to have GSH deficiency due to decreased availability of the GSH precursor amino acids glycine and cysteine. Previous studies in the elderly and HIV-infected patients found that supplementing with glycine and cysteine (provided as N-acetylcysteine, NAC) (GlyNAC) improved mitochondrial dysfunction and lowered IR; however, its effects on mitochondrial impairment or IR in T2D are unknown.

The article reports unpublished data on mitochondrial fuel oxidation, insulin resistance and free fatty acid (FFA) concentrations from a previous pilot study investigating the effect of supplementing GlyNAC in patients with T2D. The study included ten adults with poorly controlled T2D (HbA1c 8–10%) and ten non-diabetic controls. Participants with a thyroid disorder, liver or renal impairment, malignancy or consuming dietary supplements were excluded. Only newly diagnosed diabetic patients who were not receiving insulin therapy

were recruited. All diabetic participants were being treated with lifestyle modification and oral antidiabetic agents only. Diabetic patients received GlyNAC capsules formulated to provide 100mg/kg/day of glycine and 100mg/kg/day of NAC for 2 weeks. Mitochondrial fatty-acid oxidation (MFO), mitochondrial glucose oxidation (MGO), IR and FFA concentrations were assessed at baseline and 2 weeks.

There was no significant difference in BMI between the groups at baseline or after 2 weeks of GlyNAC supplementation. Compared to fasted non-diabetic controls, fasted diabetic participants had 36% lower MFO and 106% higher MGO. GlyNAC supplementation was associated with a 30% increase in MFO and a 47% decrease in MGO, indicating an improvement in impaired fasted mitochondrial fuel oxidation. Compared to non-diabetic controls, diabetic participants had severely elevated fasting insulin concentrations (160% higher) and IR (425% higher), and they decreased by 19% and 22%, respectively, after 2 weeks of supplementation with GlyNAC. FFA concentrations were also reduced by 25% in T2D patients after GlyNAC supplementation. However, there were no improvements in fasting plasma glucose concentrations.

GlyNAC supplementation did not normalise mitochondrial defects or insulin resistance to levels found in controls; this is likely due to the short 2-week duration of supplementation and the presence of uncontrolled hyperglycaemia. However, the exploratory study results are promising, with significant improvements in MFO and IR after just 2 weeks. These results could have important implications for improving health in T2D patients and support the need for larger, placebo-controlled randomised clinical trials to confirm these findings.

Multiple sclerosis and the gut mycobiome

Shah S, Locca A, Dorsett Y, Cantoni C, Ghezzi L, Lin Q, et al. Alterations of the gut mycobiome in patients with MS. EBioMedicine 2021;71:103557.

Multiple sclerosis (MS) is a pro-inflammatory demyelinating disease of the central nervous system (CNS). The exact causes are unknown, but the risk of developing MS is thought to comprise a combination of genetic and environmental factors, including an interaction between the immune system and the gut microbiota.

The gut mycobiome is the fungal component of the microbiome. It accounts for approximately 0.1% of gut microbiota and is ubiquitous in all humans. Several studies suggest mutual or competitive relationships between gut mycobiome and bacteria. Dysbiosis of the gut mycobiome has been implicated in autoimmune conditions such as irritable bowel disease, although its involvement in MS has been unstudied.

The case-control observational study characterised the gut mycobiome in MS patients (n=25) and healthy controls (n=22) at baseline and after 6 months. Participants were 18–50 years old, who had not taken disease-modifying therapy (DMT) or steroid treatments in the past 3 months and were not in a clinical relapse at study enrolment. Stool and blood samples were collected at baseline and after 6 months for the gut mycobiome and blood immune cell analyses, and a 4-day food frequency questionnaire was recorded to provide qualitative dietary information. The gut mycobiome was characterised using ITS1 (gene) sequencing. The association between the gut mycobiome community and four food groups was also assessed, including butter and animals, nuts and seeds, refined grains, and whole grains.

The mycobiome had significantly higher alpha diversity and inter-subject variation in MS patients than controls. *Saccharomyces* was over-represented in MS patients (42%) compared to controls (23%). *Aspergillus*, which forms part of the respiratory and gut mycobiome and produces aflatoxins (toxins) that can provoke opportunistic infections, was also over-represented. *Aspergillus* was positively correlated with activated CD16+ dendritic cells in MS patients. *Saccharomyces* was positively correlated with circulating basophils and negatively correlated with regulatory B cells. *Saccharomyces* have previously been shown to have protective and detrimental effects on gut inflammation and human health.

Gut fungi can directly impact the immune response or indirectly through their interactions with bacteria. In the current study, MS patients had a disrupted correlation pattern between fungi and peripheral immune profile and 1.7-fold fewer correlations between mycobiome and immune profiles than controls. Furthermore, *Saccharomyces* had a negative correlation with *Lachnospiraceae incertae sedis* in MS patients, raising the possibility that the mycobiome may affect autoimmune through the regulation of bacteria.

Food intake analysis also suggested that specific food types alter the gut mycobiome. A moderate correlation was found between butter and animal fat and the relative abundance of *Saccharomyces* and *Hannaella*. The relative abundance of both was also significantly higher in obese subjects. In MS subjects alone, there was a negative correlation between *Saccharomyces* and nuts and seeds and a stronger association between *Hannaella* and butter and animal fats. A moderate increase in *Aspergillus* abundance was linked to egg and refined grain and was stronger in the MS group.

The study is the first to analyse the interplay between diet, gut mycobiome, the immune system and metabolism and their contribution to disease pathogenesis and progression in people with MS. Further studies with a larger sample size and more longitudinal time points are required to discover the precise characteristics of the gut mycobiome dynamics and to assess the causal association of the mycobiome with MS and its direct or indirect interactions with gut bacteria and autoimmunity.

Grape powder and gut microbiome

Yang J, Kurnia P, Henning SM, Lee R, Huang J, Garcia MC, et al. Effect of standardized grape powder consumption on the gut microbiome of healthy subjects: a pilot study. Nutrients 2021;13(11):3965.

The gut microbiome plays a crucial role in host cholesterol homeostasis, including microbial cholesterol and bile acid (BA) metabolism, with changes in the intestinal environment also linked to obesity, type 2 diabetes mellitus and cardiovascular disease. Studies have shown that fruits high in polyphenols can have prebiotic effects, leading to changes in gut microbiota composition. In experimental studies, supplementation with a polyphenol-rich pomegranate extract and inulin had positive effects on cholesterol and bile acid metabolism, in association with changes in the gut microbiota. Grapes are a rich source of both polyphenols and fibre; however, there is limited information on the effects of grape consumption on the gut microbiome and cholesterol metabolism in humans. Therefore, the current study was designed to assess the impact of grape powder on the gut microbiome and cholesterol/bile acid metabolism in healthy adults.

The pilot study was a two-phase intervention study including a 4-week standardisation to a low-polyphenol diet, followed by 4 weeks of grape powder consumption (46g/day, comprising 2–3g of dietary fibre) while continuing the low-polyphenol diet. Eligibility criteria included good health, 18–55 years of age, and habitually consuming a low-fibre/low-polyphenol diet. Postmenopausal women, subjects taking antibiotics, laxatives or probiotics within the past 3 months, or any medication or dietary supplement that could interfere with polyphenol absorption were excluded. Blood, stool and urine samples were collected during both phases. At weeks 0, 4 and 8, the subjects completed and returned 3-day food records that a dietitian evaluated for compliance with the low-fibre/low-polyphenol diet.

Nineteen subjects, aged 21–55 years, completed the study. Their body mass index (BMI) and body weight were similar between baseline and the end of the study. Compared to the baseline, 4 weeks of grape powder consumption significantly increased the alpha diversity index of the gut microbiome. In addition, grape powder induced a significant increase in *Akkermansia* abundance, which is known to have positive effects on glucose levels and lipid metabolism. There were also significant increases in *Lachnospiraceae UCG-010* and *Flavonifractor*, but a decrease in *Bifidobacterium* and *Dialister* at the genus level.

After 4 weeks of grape powder consumption, the serum total cholesterol had decreased by 6.1%, and HDL cholesterol by 7.6%. There was also a trend of decreasing LDL cholesterol by 5.9% and decreasing total bile acid by 40.9%. Triglyceride levels were not changed by grape powder consumption. No significant correlation was detected between the changes in serum triglycerides, cholesterol, HDL cholesterol, LDL cholesterol and microbial phyla. However, positive correlations between bile acids and specific microbial phyla were observed.

In summary, the results provide preliminary information about the positive effects of grape powder intake on the gut microbiome, host cholesterol and BA metabolism. However, the study did produce mixed results, with a significant reduction in HDL cholesterol observed after 4 weeks of grape powder consumption and further studies are required to under the health effects of grape powder consumption.

References

Guasch-Ferré M, Li Y, Willett WC, Sun Q, Sampson L, Salas-Salvadó J, et al. Consumption of olive oil and risk of total and cause-specific mortality among US adults. Journal of the American College of Cardiology 2022;79(2):101–112.

Gutiérrez-Castrellón P, Gandara-Martí T, Abreu Y Abreu AT, Nieto-Rufino CD, López-Orduña E, Jiménez-Escobar I, et al. Probiotic improves symptomatic and viral clearance in Covid-19 outpatients: a randomized, quadruple-blinded, placebo-controlled trial. Gut Microbes 2022;14(1):2018899.

Honigberg MC, Ye Y, Dattilo L, et al. Low depression frequency is associated with decreased risk of cardiometabolic disease. Nature Cardiovascular Research 2022.

Schönenberger KA, Schüpfer AC, Gloy VL, Hasle, P, Stanga Z, Kaegi-Braun N, Reber E. Effect of anti-inflammatory diets on pain in rheumatoid arthritis: a systematic review and meta-analysis. Nutrients 2021;13(12):4221.

Sekhar RV. (GlyNAC (Glycine and N-Acetylcysteine) supplementation improves impaired mitochondrial fuel oxidation and lowers insulin resistance in patients with type 2 diabetes: results of a pilot study. Antioxidants 2022;11(1):154.

Shah S, Locca A, Dorsett Y, Cantoni C, Ghezzi L, Lin Q, et al. Alterations of the gut mycobiome in patients with MS. EBioMedicine 2021;71:103557.

Yang J, Kurnia P, Henning SM, Lee R, Huang J, Garcia MC, et al. Effect of standardized grape powder consumption on the gut microbiome of healthy subjects: a pilot study. Nutrients 2021;13(11):3965.

CPD hours

The *AJHNM*-based CPD questionnaire system is a voluntary system designed to assist members in the accumulation of NHAA CPD hours. Each question refers to an article in this issue of the *Australian Journal of Herbal and Naturopathic Medicine*. Hours accumulated through completion of these questions should be recorded in the NHAA CPD diary. The completion of answering each issue's questions qualifies for 4 hours within the Formal Learning category. Your completed CPD diary should be returned with your membership renewal at the end of the financial year. For further information, please see the NHAA CPD Guide on the NHAA website www.nhaa.org.au.

MedHerb CPD questions March 2022

With reference to the study on green tea/green tea extracts and liver biomarkers, which of the following statements is false:

- The NHANES study proved a cause-and-effect relationship between green tea extracts and abnormal liver biomarkers.
- Green tea consumption significantly reduced the odds of having one or more abnormal liver biomarkers.
- No significant association was found between green tea supplements and abnormal liver biomarkers.
- In the NHANES study, nearly a quarter of the cohort had at least one abnormal liver biomarker.

With reference to the study on ashwagandha and perimenopause, which of the following statements is true:

- Compared to placebo, ashwagandha supplementation significantly increased serum testosterone levels.
- Ashwagandha intake resulted in a statistically significant increase in serum oestradiol and follicle-stimulating hormone compared with the placebo group.
- Women in the ashwagandha group reported more mild adverse events than in the placebo group.
- Total menopause Rating Scale (MRS) scores and total menopause-specific QoL (MENQoL) scores were significantly reduced after 8 weeks of ashwagandha supplementation, compared with placebo.

With reference to the study on black cumin oil and sleep disturbances, which of the following statements is true:

- Black cumin oil for 28 days significantly improved depressive symptoms.
- Polysomnography results demonstrated significant improvement in sleep parameters after 28 days of black cumin oil supplementation; however, Pittsburgh Sleep Quality Index (PSQI) scores did not indicate an improvement.
- Cortisol levels increased significantly at the end of the study compared to baseline.
- Black seed oil improved anxiety, as indicated by improvements in Depression Anxiety Stress Scale-21 (DASS-21) and Hamilton Anxiety Rating Scale-A (HAM-A) scores.

Which statement relating to the study on a probiotic and herbal combination for acne is false:

- After 8 weeks of treatment, a significant reduction in the number of superficial inflammatory lesions was reported in the probiotic + botanical extract group (group II) only.
- Probiotic supplementation alone reduced the number of superficial lesions by 39%.
- Compared to baseline, there was a significant decrease in *Cutibacterum acnes* in the three treatment groups at weeks 4 and 8.
- Compared to baseline at week 8 there were statistically significant decreases in mean squamation score and sebum secretion rate in the probiotic + herbal extract group (group II).

Which statement relating to the study on *Vitex agnus-castus* (VAC) and *Salvia officinalis* extracts on serum lipids in postmenopausal women is true:

- Greater improvements in total cholesterol levels were observed in women taking VAC compared to *S. officinalis*.
- Compared to baseline, triglyceride levels decreased in both the VAC and *S. officinalis* groups.
- In the placebo group, triglyceride levels increased over the 3-month trial period.
- Compared to baseline mean high-density lipoprotein levels decreased in both the VAC and *S. officinalis* groups.

MedJourn CPD questions March 2022

With reference to the study on olive oil consumption and mortality risk, which of the following is true:

- Compared to individuals with low olive consumption, those with the highest intake had a 29% lower risk of cardiovascular mortality.
- From 1990–2010, the average olive oil consumption increased by 5g per day, while the average margarine consumption only decreased by 2g per day.

- The pooled hazard ratio for all-cause mortality among participants reporting the highest consumption of olive oil was 0.81 compared to participants who rarely or never consumed olive oil.
- Substituting 10g per day of margarine, butter, mayonnaise and dairy fat with the equivalent amount of olive oil lowered the risk of all-cause mortality by 34%.

With reference to the study on probiotic supplementation and COVID-19, which of the following is false:

- The significant remission rate in the probiotic group was associated with an increase in microbial diversity on day 30.
- On day 30, the remission rate in the probiotic group (53.1%) was significantly higher than in the placebo group (28.1%).
- Median time to overall symptom resolution was five days shorter in the probiotic group than in the placebo group.
- Probiotic supplementation reduced nasopharyngeal viral load, lung infiltrates, hs-CRP and D-dimer on day 15.

With reference to the study on multiple sclerosis and the gut mycobiome, which of the following is true:

- The gut mycobiome, which is the fungal component of the microbiome, accounts for 10% of the total gut microbiota in humans.
- In healthy controls, the mycobiome had significantly higher alpha diversity than in MS patients, which is indicative of better gut health.
- MS patients had a higher abundance of *Saccharomyces* and *Aspergillus* compared to healthy controls.
- Specific food groups were found to impact the mycobiome in MS patients, with the intake of seeds and grains positively associated with *Saccharomyces* and *Hannaella*.

HERBAL MEDICINE SUMMIT 2022

"Plant medicine for the people"

SOFITEL SYDNEY WENTWORTH - NSW

Find out more & book at nhaa.org.au

Proudly brought to you by

Engage
NHAA EVENTS