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Article

Evaluation of Endophytic Fungal Extracts of *Mucuna pruriens* for the Presence of L-Dopa

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Abstract

Background: *Mucuna pruriens* (Fabaceae) is a natural source of levodopa (L-Dopa). Urbanization and overexploitation have made the plant scarce. Thus, investigations for alternative sources for natural L-Dopa are important. This study aimed to investigate the capability of the endophytic fungi present in *M. pruriens* plant for the production of L-Dopa. **Methods:** Compounds of air-dried plant parts were extracted with methanol. Endophytic fungi in fresh plant parts were isolated and grown to obtain metabolites. Plant extracts and the endophytic fungal metabolites were analyzed for the presence of L-Dopa with respect to the standard using thin-layer chromatography. **Results:** Nine endophytic fungi were isolated from different plant parts. Seed and pod extracts and their endophytic fungal metabolites contained L-Dopa. **Conclusion:** *M. pruriens* associated endophytic fungi could be excellent sources of natural L-Dopa.

Keywords: *Mucuna pruriens*; L-Dopa; Endophytic fungi; Parkinson's disease

Introduction

Medicinal plants are used extensively as a bioresource of natural products which used directly or indirectly in treating diseases. Demand for medicinal plants is increasing around the world as 80% of the total population in developing countries depend on medicinal plants for their primary healthcare (1). The extinction of medicinal plant species could result from increased use and overharvesting. Therefore, it is important to obtain the values of medicinal plants without disturbing their population and habitats. Nowadays, the focus of new drug sources has been shifted from plants to endophytes as endophytes that reside inside these plants are capable of producing compounds similar to the host plant (2). Therefore, endophytes present in medicinal plant species are alternative approaches for producing medicinal plant-derived biologically active substances (3).

Endophytes are a diverse group of microorganisms (4) that live below the epidermal cell layers of the tissues of plant parts without causing any negative consequences to the host plant. There can be one or more endophytes in an individual plant. The symbiotic relationship between the endophytes and their host plant stimulates the production of secondary metabolites of the host plant. The host plant can use these compounds for different purposes, including defense against pathogens and motivating plant growth. These compounds could be useful in the drug discovery process (5). Both fungi and bacteria live as endophytes, whereas fungi are the most often isolated endophytes. It has been found that endophytic

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fungi are a promising source for generating metabolic products which can be used as drugs or function as lead structures in synthetic modifications (6).

Mucuna pruriens is a medicinal plant that belongs to the family Fabaceae. Its vernacular names are Cowitch/Velvet bean (English), '*Wanduru Me'* (Sinhala), and '*Punaikkali'* (Tamil) (6). It has been shown that all parts of the plant possess medicinal values, thus exerting a broader spectrum of pharmacological activities (7). *M. pruriens* is reported to have the highest content of natural L-Dopa (8). Due to nutritional, pharmaceutical, and cosmeceutical bioactive constituents (9) present in *M. Pruriens,* the global demand for *M. pruriens* is increasing.

L-Dopa is commercially synthesized from vanillin and hydantoin by a chemical process that involves eight steps. It is a time-consuming and very expensive process that involves several chemicals. Therefore, scientists have focused on plant-derived L-Dopa (10). Sharma *et al*. (2019) have isolated thirty-five different endophytic fungal species from different parts of *M. pruriens* plants collected at eight different geographical locations in India (11). However, endophytic fungi have not been isolated from the pods and seeds of *M. pruriens* plant. It was reported that the isolation of endophytic fungi from pods and seeds of *M. pruriens* plant is difficult (11).

Therefore, the current study was undertaken to isolate endophytic fungi from different parts of *M. pruriens* plant and screen the extracts of different parts of the plant and endophytic fungi isolated from different parts of the plant for the presence of L-Dopa.

Materials and Methods

Plant materials

Leaves, seeds and pods of *M. pruriens* plant were collected from a home garden in Gampola, Central Province, Sri Lanka. A herbarium sheet was prepared, and the plant was authenticated by the National Herbarium, Royal Botanical Gardens, Peradeniya, Sri Lanka.

Chemicals, reagents and solvents

Methanol, acetone, chloroform, 1-butanol, glacial acetic acid, ethyl acetate, ethanol, ninhydrin, iodine, silica gel, and dextrose used in the study were of analytical grade. Commercially available L-Dopa tablets (TIDOMET FORTE, Torrent PHARMA, India) were purchased from University Rajya Osusala, Peradeniya, Sri Lanka.

Other materials

TLC plates (Sigma) were purchased from Aldrich, Germany. Chlorex (CLOROX, Malaysia) and potatoes were purchased from a commercial food stall in Peradeniya, Sri Lanka.

Equipment

Grinder (Waring, USA) Rotary evaporator (Buchi, India), Laminar flow cabinet (SRCJ.15), Autoclave (ALP, Japan), Water bath (Bioer, China), Centrifuge (Gemmyco, Taiwan), UV illuminator (VL-4.Lc, France), Sonicator (MRC, China), Shaker (Memmert, Germany), Analytical balance (VWR, Italy) were the equipment used in the study.

Separation of L-Dopa and carbidopa from commercially available L-Dopa-carbidopa tablets

L-Dopa-carbidopa tablets (TIDOMET FORTE, Torrent PHARMA, India) were weighed and crushed to obtain a fine powder. The crushed powder was then dissolved in a mixture of water:methanol (7:3 V/V ((Nail and Vega, 2007)), 10 ml), followed by sonication for 20 min. The solution was

centrifuged at 25,000 rpm for 15 min. The supernatant was separated from the precipitate. The solvent of the supernatant was evaporated at 45 ºC using a rotary evaporator. Obtained solid residue (considered as standard L-Dopa-carbidopa) was transferred into a clean universal bottle and stored in a refrigerator (2 – 8 ℃) until used in TLC analysis.

Selecting a suitable mobile phase

TLC was developed for the standard L-Dopa-carbidopa and methanolic extract of seeds using acetone:chloroform:1-butanol:glacial acetic acid:water in volume based solvent ratios; 6:4:4:4:3.5 (12) and 6:4:4:4:3.8. After the development, plates were sprayed with ninhydrin. TLC patterns and Rf values were compared to select the best mobile phase.

Preparation of plant extracts (Direct extraction)

A part of the collected plant parts was shade dried for four weeks inside a room and processed separately for direct extraction. Dried plant samples (leaves, seeds, and pods) were ground separately using a mechanical grinder to obtain coarse powders of respective plant parts and processed separately. Powdered plant materials (each 25 g) were sonicated with methanol (200 ml) and filtered through Whatman filter paper. The filtrate was collected. The procedure was repeated twice for the solid residue. Filtrates were combined. The solvent of the resulting extract was evaporated to dryness under pressure at 40 ℃ using a rotary evaporator. Crude solid extracts were stored in the freezer until they were used.

Preparation of endophytic fungal extracts

Isolation and growth of endophytic fungi

Fresh plant samples (leaves, seeds, and pods) were subjected to surface sterilization by the following procedure. Initially, plant materials were washed several times under running tap water, followed by washing in sterile distilled water. Surface sterilization was then carried out by subsequently rinsing the plant materials with 70% ethanol for 30 seconds, then with chlorex for 2–3 min, followed by rinsing in 70% ethanol for nearly 2 min, and finally with sterile distilled water for 2 minutes. Plant samples were then dried between folds of sterilized steri tissue papers. Then, surface sterilized plant samples were cut into small segments (approx. 5 mm×5 mm) using a sterile scalpel. Small segment samples were placed on Petri dishes containing potato dextrose agar (PDA) supplemented with gentamicin (320 µg/ml). Plates were then labeled properly and incubated at room temperature inside a clean box until fungal growth was initiated. When the fungal growth was initiated, another two days were allowed for further growth. Using a sterile cork-borer, growing tips of fungal mycelia were punched from the agar plate and transferred to a new PDA plate supplemented with gentamicin (320 µg/ml) for pure culture. After that two new PDA plates were inoculated from each pure fungal culture, and incubated in the dark at room temperature for two weeks.

Preparation of fungal extracts

New pure fungal cultures on PDA plates were cut into small segments using a scalpel and transferred to a sterile 250 ml Erlenmeyer flask containing potatoes dextrose broth (PDB) (150 ml). One Erlenmeyer flask containing PDB was kept without adding any fungal extracts. The mouth of each Erlenmeyer flask was quickly closed with a pre-sterilized cotton plug. Then the neck of the flask and the cotton lid was covered with a pre-sterilized aluminium foil. These starter endophytic fungal cultures were then placed on an orbital shaker (shaking at 120 rpm for 20 min). After that, they were removed from the shaker and allowed to continue growing in the dark for two weeks with intermittent shaking. The content of each Erlenmeyer flask was filtered through a funnel with a Whatman filter paper followed by a collection of filtrates. Filtrate (aqueous) from each Erlenmeyer flask was partitioned with ethyl acetate (100 ml). Organic and aqueous phases were evaporated separately under vacuum at 40 ºC and 44 ºC, respectively.

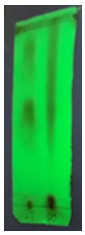
TLC analysis

TLC was developed for the solid extracts of plant materials (leaves, seeds, and pods); organic and aqueous phases of endophytic fungal extracts; and standard L-Dopa-carbidopa using acetone:chloroform:1-butanol:glacial acetic acid:water (6:4:4:4:3.8 V/V/V/V/V) as the mobile phase. Air dried TLC plates were analyzed by three methods. They were observed under UV light (254 nm) applied with ninhydrin (0.5%) in methanol and kept in an iodine chamber to visualize the spots. Retardation factor (Rf)s of solid extracts of plant materials (leaves, seeds, and pods) and endophytic fungal extracts were compared with standard L- Dopa-carbidopa to identify any similarities.

Results

Table 1 shows the results of the TLC analysis carried out to evaluate the presence of L-Dopa in methanolic extracts of leaves, seeds, and pods of *M. pruriens* with respect to standard L-Dopa.

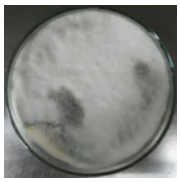
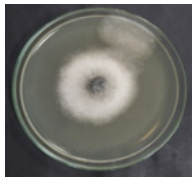
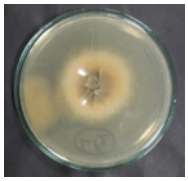
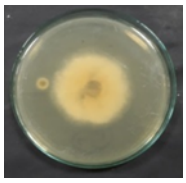
Table 1. Results of TLC analysis on methanolic extracts of leaves, seeds and pods of *M. pruriens*

***M. pruriens* plant extract**	**TLC plate observed under UV light, 254 nm***	**TLC plate after spraying ninhydrin and drying***	**TLC plate after vaporizing iodine***
Leaf extract			
Seed extract			
Pod extract			

*The left most spot in each TLC plate was of the standard L-Dopa-carbidopa

A total of nine endophytic fungal species were isolated from *M. pruriens* plant materials. Among the nine endophytic fungi, three were isolated from leaves (LF1-LF3), five were from seeds (SF1-SF5), and one was from pods (PF) and shown in Tables 2-4, respectively.

Table 2. Endophytic fungi from leaves of *M. pruriens*

Leaf fungi	Front view	Back view	Streak plate
LF1			
LF2			
LF3			

LF1, 1st leaf fungus; LF2, 2nd leaf fungs; LF3, 3rd leaf fungus

Discussion

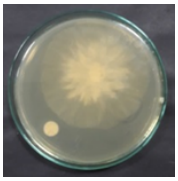
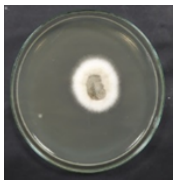
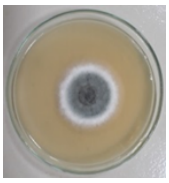
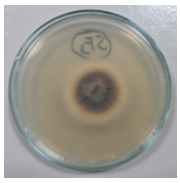
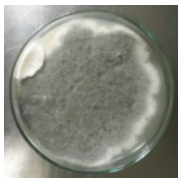
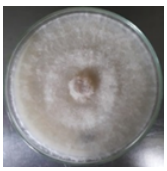
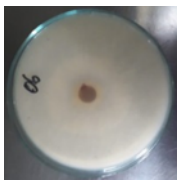
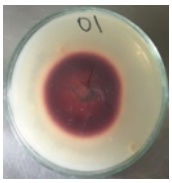
The unavailability of standard L-Dopa was rectified using the commercially available L-Dopa-carbidopa in 10:1 w/w ratio, respectively in tablet dosage form Acetone:chloroform:1-butanol:glacial acetic acid:water (6:4:4:4:3.8 V/V/V/V/V) solvent system was chosen as the mobile phase due to the better separation observed for the L-Dopa and carbidopa. A quantitatively larger spot with a 0.54 Rf value was obtained for L-Dopa, whereas carbidopa Rf was 0.84, which was a clear distinction from that of L-Dopa.

The presence of L-Dopa in the seeds of *M. pruriens* has been evaluated by several study groups (8, 9, 13). The present study also confirmed the presence of L-Dopa in seeds of *M. pruriens* regardless of geographical differences. According to the results shown in Table 1, methanolic extracts of seeds and pods obtained a dark blue spot. The respective Rf values were matched exactly with the standard L-Dopa (Rf, 0.54) spot. In comparison, a dark blue/Ruhemann's purple spot did not appear in the methanolic extract of leaves. The findings of this study imply that the dried seeds and pods of the *M. pruriens* plant contain L-dopa, whereas L-Dopa is not present in the leaves of *M. pruriens* plant. It has been reported that fresh leaves of *M. pruriens* plant contain 1 % of L-Dopa (13). However, there were no records on L-Dopa isolated from the pods of the *M. pruriens*.

Endophytic fungi isolated from leaves, seeds and pods of *M. prriens* were not identified but separately screened for the production of L-Dopa. For that, well grown fungi broths were separately extracted to organic and aqueous solvents.

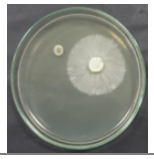
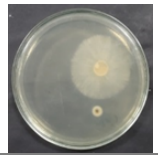
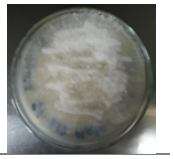
The broths containing SF1 and SF5 fungal cultures turned yellow to orange-red. This could be due to the increased broth incubation period, production of tyrosinase enzyme lead to the conversion of L-Dopa into melanin (14). As a result, the yield of L-Dopa could be low. Further studies are needed in this area for confirmation of the proposed idea.

Table 3. Endophytic fungi from seeds of *M. pruriens*

Seed fungi	Front view	Back view	Streak plate
SF1			
SF2			
SF3			
SF4			
SF5			

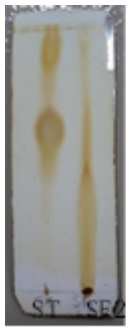
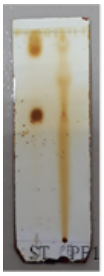
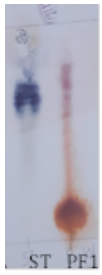
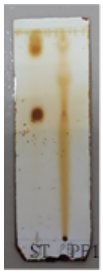
SF1, 1st seed fungus; SF2, 2nd seed fungus; SF3, 3rd seed fungus; SF4, 4th seed fungus; SF5, 5th seed fungus; SF6, 6th seed fungus

Table 4. Endophytic fungi from pods of *M. pruriens*

Pod fungus	Front view	Back view	Streak plate
PF			

PF, Pod fungus

Table 5. TLC analysis of water fraction of the SF1, SF2, PF endophytic fungal extracts

Endophytic fungal extract	TLC plate observed under UV light, 254 nm*	TLC plate after spraying ninhydrin and drying*	TLC plate after vaporizing iodine*
SF1			
SF2			
PF			

*The left most spot in each TLC plate was of the standard L-Dopa-carbidopa; SF1, 1st seed fungus; SF2, 2nd seed fungus; PF, Pod fungus

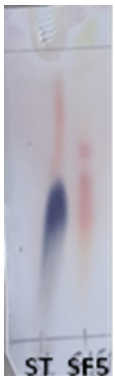


Figure 1. TLC analysis of ethyl acetate fraction of SF5 endophytic fungal extract of *M. pruriens* plant (The left most spot in the TLC plate was of the standard L-Dopa-carbidopa)

Furthermore, a previous study reported that the seeds of *M. pruriens* plant possess Proline as a pharmaceutical constituent. It may be assumed that SF1 and SF5 fungi produce proline or hydroxyl proline as one of the spots was visually observed as yellow after ninhydrin spraying and drying. However, no firm conclusion could be drawn due to the limited data available. As ninhydrin reacts with an amine functional group of alpha-amino acids to form purple-coloured compounds, Only L-Dopa gives a purple-colored compound. Carbodopa does not produce a purple-coloured compounds with ninhydrin. Therefore, It can be concluded that the purple-coloured compound is L-Dopa.

According to the results shown in Table 5, low-intensity blue/Ruhemann's purple colour spots were obtained on TLC plates with water fractions of SF1, SF2 and PF fungal extracts. A similar spot was obtained by ethyl acetate fraction of SF5 fungal extract, as shown in Figure 1. This reveals that endophytic fungi isolated from the seeds and the pods of *M. pruriens* produce low-yield L-Dopa.

Conclusion

The dried seeds and pods of *M. pruriens* plants could be a potential sources of natural L-Dopa. The findings provide the evidence that endophytic fungi present in the *M. pruriens* plant produce L-Dopa similar to those found in the host plant.

Author Contributions: Conceptualization, CPTL; methodology, CPTL, YS; analysis, YS; investigation, CPTL, YS; writing—original draft preparation, YS; writing—review and editing, CPTL; supervision, CPTL; project administration, CPTL. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

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Article

Use and Attitudes of Complementary and Alternative Medicines Among Allied Health Sciences Students, University of Peradeniya

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Abstract

Background: Complementary and Alternative Medicine (CAM) consists of a group of medical and health care systems, practices, and products delivered outside the mainstream health care system. CAM treatments can be used not only to treat diseases but also to promote wellness, disease prevention, and manage symptoms. However, someone's attitudes toward health may contribute to seeking CAM treatments. This study aimed to investigate the use and attitudes of CAM among Allied Health Sciences (AHS) students at the University of Peradeniya. **Methods:** An online questionnaire was used and all AHS students at the University of Peradeniya were invited to participate. The questionnaire comprised three parts: Part A) demographic data, Part B) usage of complementary and alternative medicines and Part C) attitudes toward complementary and alternative medicines. CAM usage and attitude differences between different degree programs and academic years were compared. **Results:** Of the 601 students invited, 314 (52.2%) responded to the questionnaire. Among them, 143 participants (45.5%) had used CAM during the past 12 months. Of them, vitamins were the most used CAM. Most participants (70.1%) had used CAM to improve their health, while (68.5%) had selected CAM to reduce side effects. More than one-third of students showed positive attitudes towards CAM. There were significant differences in CAM usage between students of pharmacy/nursing and MLS/Radiography/Physiotherapy, while there was no difference in attitudes regarding CAM. There were differences in attitudes concerning CAM between senior and junior students, however no difference in CAM usage. **Conclusion:** This study found that AHS students commonly reported using CAM. A comparison between Pharmacy/Nursing and MLS/Radiography/Physiotherapy students suggests that pharmacy/nursing education is associated with improved awareness and use of CAM among AHS undergraduate students in a Sri Lankan university.

Keywords: Complementary and Alternative Medicines; Usage, Attitudes, University Students

Introduction

Complementary and Alternative Medicine (CAM) consists of a group of medical and health care systems, practices, and products delivered outside the mainstream health care system (1). They are delivered by a trained practitioner or administered as a self-care practice and have a heterogeneous spectrum that includes ancient to modern approaches to prevent or treat diseases. Complementary medicines are treatments used along with allopathic medicines (Massage therapy, Vitamins), whereas alternative medicines are used instead of allopathic medicines (Unani. Siddha) (2,3). Treatment procedures of allopathic medicine are based more on evidence-based approaches, which refer to the distinct and precise

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use of current evidence in making decisions about the treatment and care of patients (4). In contrast, CAM decision-making approaches are mainly based on experience, observation, and traditional healing manuscripts. As a general term, CAM can include all sorts of practices and modalities that are used in agreement with its literal description. But their effectiveness, approval for medical use, and the evidence of their benefit or harm can vary widely (3).

In both developed and developing countries, CAM is commonly practiced (5, 6), and its usage and popularity have increased gradually over time (4, 6). It has been used to improve health and well-being and treat or prevent many diseases and conditions. Dissatisfaction with allopathic medicine is also a reason for seeking CAM treatment by patients because of poor patient-physician communication and lack of time spent with the physician. Additionally, someone's attitudes toward health may also contribute to the decision to seek CAM treatments. Having a philosophical orientation and having greater control over personal health also affects the selection of CAM treatments (7). In addition, the rapid increase of interest in CAM in academic, industrial, and economic sectors contributes to its high prevalence of use (8). The acceptability, choice, and decision-making on CAM depend on many reasons, such as the availability of CAM, the belief in the safety of CAM, unsatisfactory results from mainstream therapies (7), and dissatisfaction with allopathic medicines (7,9). In developing countries, CAM therapies are mainly performed by people who do not have academic training in this field. It may increase the risk of deviation from scientific approaches and eventually cause unwanted complications. Therefore, medical students' awareness of CAM and perhaps how to deal with patients who simultaneously use CAM and the usefulness of CAM for patients in certain circumstances can be discussed at the university level.

Ease of availability and perceived efficacy of natural approaches are primary factors of preference toward CAM (10). A previous study reported that most students in Allied Health sciences believe CAM is based on ideas and methods from which allopathic medicine can benefit. Further, pharmacy and nursing students believe that CAM will be more beneficial after their studies to apply in real-world scenarios (11, 12). The research findings can be used to generate further research questions and inform academics on how to discuss CAM with students regarding important aspects. As future healthcare professionals, today's Allied Health students should have adequate knowledge of CAM. From the findings on the use and attitudes about CAM among Allied Health students, this study may help identify Allied Health students' perception of CAM and make necessary decisions to improve the knowledge of Allied Health students regarding CAM.

Some studies argued that the most influential factor in patients' decision on CAM may be perceived efficacy (13). Also, the literature suggests that better use of CAM may be beneficial to reducing the load on overburdened health systems, especially in developing countries. This would require incorporating some discussions regarding CAM in universities' medical and Allied Health Science Faculties (4). However, this curriculum for CAM should be developed based not only on opinions but also on scientific data (5). In this study, we aimed to investigate the use and attitudes of Complementary and Alternative Medicines among Allied Health Sciences students at the University of Peradeniya.

Materials and Methods

Study design and setting

All undergraduate students (Pharmacy, Nursing, Medical Laboratory Sciences, Radiography, and Physiotherapy students) of the Faculty of Allied Health Sciences (FAHS), University of Peradeniya, were invited to participate in this descriptive cross-sectional study.

Data collection tool

An online questionnaire (a Google form) was used to collect data from the study participants. The questionnaire for this study was developed using previously validated questionnaires (4,10,14,15). The content and format of the questionnaire were evaluated using a pre-test involving a convenience sample of 20 students, and they were not included in the final analysis. The online questionnaire was in English. It consisted of three main sections, Part A: Demographic data, Part B: Usage of Complementary and Alternative Medicines (12 questions), and Part C: Attitudes on Complementary and Alternative Medicines (11 statements). All questions were close-ended questions.

Inclusion and exclusion criteria

All first, second, third- and fourth-year Sri Lankan undergraduates from the Faculty of Allied Health Sciences, University of Peradeniya, who provided informed consent, participated in the study. Students who were involved in the pre-test were excluded from the main analysis.

Data analysis

Data analyses were performed using Statistical Package for Social Sciences (SPSS) software version 26. Data were summarized using tables and graphs and expressed as frequencies. Participants were grouped into two based on their undergraduate degree programs, Group 1 – 'Nursing and Pharmacy students' and Group 2 – 'Medical Laboratory Science, Physiotherapy, and Radiography students'. Further, the students were categorized into another two groups: junior students – 'first and second-year students' and senior students – 'third-year and fourth-year students'. Categorical variables between different degree programs and different academic years were compared using the Chi-Square test. A P value < 0.05 was considered as significant.

Ethical consideration

Before commencement of the study, ethical approval was obtained from the Ethics Review Committee, Faculty of Allied Health Sciences, the University of Peradeniya (Ethics Approval Number - AHS/ERC/2021/005).

Results

Demographic data

Data collection was done from the 10th of May to the 10th of June 2021. The online questionnaire was sent to all students with a valid email address (n=601) and registered at the Faculty of Allied Health Sciences, University of Peradeniya. The total response rate was 52.2% (314 students). When considering the academic year, most responses were from the fourth year (2015/2016 batch), which was 15.8% out of the total number of responded participants. Second-year students showed a lower response rate of about 8.6%. When considering the degree program of the participants, the Department of Nursing showed a higher response rate, about 12.3% out of all the participants. The lower response rate was 7.6% among the Department of Radiography participants. Most study participants were female students (Tables 1 & 2).

Awareness of Complementary and Alternative Medicines

Two-thirds of participants (66.6%) were aware of the term Complementary and Alternative Medicines, while the rest (33.4%) were not. However, only 42.7% of participants were aware of the difference between Complementary and Alternative Medicines, while 57.3% were not.

Table 1: Students' response rate for the survey questionnaire

	No. of students invited to participate	No. of responded students (Percentage %)	Percentage % by the total
Academic year			
1st Year	187	82 (43.8)	13.6
2nd Year	172	52 (30.2)	8.6
3rd year	140	85 (60.7)	14.1
4th year	122	95 (77.8)	15.8
Total	601	314	52.2
Degree program			
MLS	102	63 (61.7)	10.4
Nursing	151	74 (49.0)	12.3
Pharmacy	100	68 (68)	11.3
Physiotherapy	107	63 (58.8)	10.4
Radiography	141	46 (32.6)	7.6
Total	601	314	52.2

Table 2: Socio-demographic data of the students of the Faculty of Allied Health Sciences by the academic year

	1st year (2018/2019 batch) (%)	2nd year (2017/2018 batch) (%)	3rd year (2016/2017 batch) (%)	4th year (2015/2016 batch) (%)
Field of study				
MLS	12 (3.8)	18 (5.7)	11 (3.5)	22 (7.0)
Nursing	23 (7.3)	13 (4.1)	13 (4.1)	25 (7.9)
Pharmacy	28 (8.9)	8 (2.5)	15 (4.7)	17 (5.4)
Physiotherapy	11 (3.5)	3 (0.9)	34 (10.8)	15 (4.7)
Radiography	8 (2.5)	10 (3.1)	12 (3.8)	16 (5.0)
Gender				
Female	59 (18.7)	41 (13.0)	66 (21.0)	74 (23.5)
Male	23 (7.3)	11 (3.5)	19 (6.0)	21 (6.6)
Nationality				
Sri Lanka	82 (26.1)	52 (16.5)	85 (16.5)	95 (30.2)
Other	0	0	0	0

Use of Complementary and Alternative Medicines

Among the participants, 143 (45.5%) used some form of CAM during the past 12 months, while 171 (54.5%) did not. Vitamins, Herbal medicines, and Ayurveda were the most commonly used CAM among all participants, while Homeopathy, Unani, Siddha, and Acupuncture were the least used CAM types. Vitamins were used by 60.9% of participants, and among them, the majority were fourth-year students. Herbal medicines were used by 48.9% of the participant, with the majority among first-year students (53.7%). The third primarily common CAM was Ayurveda. A high prevalence was observed among the

first-year students (46.3%) and less prevalence in the second-year. Among all the participants in this study, only three students used Acupuncture, Siddha, and/or Unani CAM.

A high number of first-year students have used vitamins, which was about 63.4%, while no one was using Acupuncture, Homeopathy, and Siddha. Among second-year students, the majority used Herbal medicine (68.2%) and vitamins (50.0%). Ayurveda and Herbal medicines were used by a similar number of students from the third-year. Most third-year students have used Vitamins (50.0%). The most commonly used CAM among fourth-year students was Vitamins, which was about 72.2%. Lesser number of students in all years practiced Yoga, Acupuncture, Homeopathy, Siddha, and Unani (Table 3).

Table 3: Usage of Complementary and Alternative Medicines during the last 12 months among undergraduate students of the Faculty of Allied Health Sciences by the academic year

	First-year (2018/2019) (%)	Second-year (2017/2020) (%)	Third-year (2016/2021) (%)	Fourth-year (2015/2016) (%)	Total Frequency (%)
CAM Type					
Acupuncture	0 (0.0)	0 (0.0)	1 (2.9)	0 (0.0)	1 (0.7)
Ayurveda	19 (46.3)	7 (31.8)	12 (35.3)	11 (25.0)	49 (34.7)
Herbal medicine	22 (53.7)	15 (68.2)	13 (38.2)	19 (43.2)	69 (48.9)
Homeopathy	0 (0.0)	1 (4.5)	1 (2.9)	0 (0.0)	2 (1.4)
Massage	8 (19.5)	3 (13.6)	7 (20.6)	4 (9.1)	22 (15.6)
Meditation	14 (34.1)	5 (22.7)	8 (23.5)	11 (25.0)	38 (26.9)
Music therapy	4 (9.8)	2 (9.1)	6 (17.6)	5 (11.4)	17 (12.0)
Siddha	0 (0.0)	0 (0.0)	1 (2.9)	0 (0.0)	1 (0.7)
Unani	1 (2.4)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.7)
Vitamin	26 (63.4)	11 (50.0)	17 (50.0)	32 (72.2)	86 (60.9)
Yoga	2 (4.9)	2 (9.1)	0 (0.0)	2 (4.5)	6 (4.2)
Other	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	41	22	34	44	141

Among all participants, CAM was used by a higher number of participants to improve health, (37.7%). Among them, fourth-years have reported a higher prevalence. Few participants used CAM only to prevent illness (6.2%). Among them, second-years showed a poorer response than the other three years (4.5%). Most participants selected CAM only to reduce harm to the body (30.6%), while fourth-years showed a higher response (36.6% compared to the other three years).

There was a significant difference in the use of all types of CAMs investigated in this study in the two groups of students: Group 1 and Group 2 students ($p < 0.05$). Comparatively, all CAM types such as Herbal medicines, Meditation, Music therapy, and Vitamin usage were higher in group 1 (Table 4).

Table 4: Difference in usage of Complementary and Alternative Medicines between Group 1 and Group 2

	Group 1 (n=164) Frequency (%)	Group 2 (n=128) Frequency (%)	p*
CAM Type			
Ayurveda	26 (15.8)	23 (17.9)	0.011
Herbal medicines	40 (24.3)	29 (22.6)	0.006
Massage	12 (7.3)	10 (7.8)	0.008
Meditation	25 (15.2)	13 (10.1)	0.002
Music therapy	12 (7.3)	5 (3.9)	0.003
Vitamin	45 (27.4)	41 (32.0)	0.008

* Chi-square test. Some students have used more than one CAM type. Therefore, the total percentage does not add up to 100%. Group 1 – Nursing and Pharmacy students, Group 2 – Medical Laboratory Science students, Physiotherapy students, and Radiography students.

Usage of all CAM types investigated in this study was not significantly different by two student groups: 'first and second-year students and 'third-year and fourth-year students ($p > 0.05$). (Table 5)

Table 5: Difference in usage of Complementary and Alternative Medicines between junior and senior students.

	first and second-year students (n=142) Frequency (%)	third and fourth-year students (n=150) Frequency (%)	p
CAM Type			
Ayurveda	26 (18.3)	23 (15.3)	0.274
Herbal medicines	37 (26.0)	32 (21.3)	0.077
Massage	11 (7.7)	11 (7.3)	0.735
Meditation	19 (13.3)	19 (12.6)	0.618
Music therapy	6 (4.2)	11 (7.3)	0.635
Vitamin	37 (26.0)	49 (32.66)	0.716
Yoga	4 (2.8)	2 (1.3)	0.443†

* Chi-square test. Some students have used more than one CAM type. Therefore, the total percentage does not add up to 100%. †Fisher's exact test p value.

Attitudes towards Complementary and Alternative Medicines

The majority of the participants thought that 'CAM built up the body's own immunity and promoted self-healing' (70.1%) 'CAM have less side effects (68.5%), 'CAM have less complications when taking them' (63.7%), and 'taking CAM therapies was not harmful' (55.1%). However, most of the student-participants did not have an idea about the statement 'the results of CAM are in most cases due to a placebo effect' (58.9%) and 'CAM have better healing power than allopathic medicines' (51.9%). In comparison, the highest level of disagreement was obtained for 'CAM is a threat to public health' (55.7%) followed by 'traditional medicine practices performed by non-physicians are acceptable' (38.5%) (Table 6).

Table 6: Attitudes toward Complementary and Alternative Medicines in undergraduate students of the Faculty of Allied Health Sciences

Attitude statement about CAM	Agree Frequency (%)	Disagree Frequency (%)	No idea Frequency (%)
There are less side effects when taking CAM	215 (68.5)	25 (8.0)	74 (23.6)
There are less complications when taking CAM	200 (63.7)	27 (8.6)	87 (27.4)
CAM builds up the body's own immunity and promotes self-healing	220 (70.1)	17 (5.4)	77 (24.5)
Taking CAM therapies is not harmful	173 (55.1)	43 (13.7)	98 (31.2)
CAM have better healing power than allopathic medicines	83 (26.4)	68 (21.7)	163 (51.9)
The results of CAM are in most cases due to a placebo effect	74 (23.6)	55 (17.5)	185 (58.9)
It is necessary to instruct CAM subjects in the health curriculum in universities	215 (68.5)	24 (7.6)	75 (23.9)
Physicians who practice CAM must be licensed	200 (63.7)	30 (9.6)	84 (26.8)
Traditional medicine practices performed by non-physicians are acceptable	80 (25.5)	121 (38.5)	113 (36.0)
CAM is only effective in treating minor complaints and ailments	92 (29.3)	86 (27.4)	136 (43.3)
CAM is a threat to public health	56 (17.8)	175 (55.7)	83 (26.4)

The majority of group 2 students agreed to the statement 'there are less side effects when taking CAM' when compared to group 1 students, but no difference was observed [Group 1 (31.2%), Group 2 (37.3%)]. Although there was a difference between the two groups regarding their attitude on 'There are less complications when taking CAM', there is no significant difference.

The majority of senior students agreed with the statement, 'There are less complications when taking CAM' than the junior students', and there was a significant difference between these two groups [junior students (23.2%), senior students (40.4%), $p = 0.008$].

Discussion

CAM treatments can be used to treat diseases and promote wellness, disease prevention, and manage symptoms (e.g. pain, insomnia, and hot flash) (9). Evidence from the literature suggests that some chronic diseases that are not responding well to allopathic medicines may respond to certain CAMs (7). Some examples are people with HIV who respond to herbal, mineral, and vitamin supplements (16), patients with inflammatory bowel disease who respond to herbs and botanicals (17), and people with terminal cancer who responds to detoxification with coffee and a special diet cleanser and vitamins, enzymes, and minerals (18).

At the time of investigation, this study found that nearly half of the students used some type of CAM during the last 12 months, while most of them used Vitamins, Herbal medicines, and Ayurveda. Most of them reported positive attitudes regarding CAM. The results also indicate that most students were aware of the term CAM. However, most participants reported a lack of understanding of the terms Complementary and Alternative and relating these terms with medicine. There was no difference between the use of CAM or attitudes towards CAM among senior and junior students.

Interestingly, the use of CAM was different among Group 1 and Group 2. This highlights that the pharmacy and nursing undergraduate education may be one reason that contributes to greater awareness of different CAM types and, therefore, their use. This could likely be due to the inclusion of subjects such as pharmacology and social pharmacy that discusses other therapies that patients might use, their potential interactions with allopathic treatment and how it fits into their broader treatment-seeking behaviour.

Previous studies on attitudes and beliefs of CAM reported that users of CAM believe it is safer than allopathic medicines. Moreover, they think CAM is a holistic approach to maintaining personal health and well-being (10). However, some patients consider it a supplement rather than a substitute for allopathic medicine (7,11). There are positive attitudes among the general population. Therefore, this may lead to encouraging a positive reframing for particular CAM therapies (9,10) than allopathic medicines, especially among patients with chronic illnesses (10) like cancer (9).

In this study, we approached all undergraduate students in all five departments of the Faculty of Allied Health Sciences. The questions included in this study were pre-tested and used in previous studies, making the questionnaire more reliable in comparing results with similar international studies.

There are a few potential limitations of this study. The response rate was comparatively lower probably due to the many online surveys received by university students during the COVID19 pandemic. Furthermore, several emails bounced back when sending them due to incorrect email addresses.

Conclusion

In conclusion, the findings of this study provide an understanding of CAM use among AHS students in a Sri Lanka university. Awareness of CAM was no different among senior AHS students compared to junior AHS students. This study also showed that pharmacy/nursing education is associated with improved knowledge about CAM compared to MLS/Radiography/Physiotherapy students. These gaps in student knowledge can be overcome by introducing a collaborative education plan for all AHS undergraduates.

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Article

Evaluation of Patient Related Factors, the Level of Glycemic Control; and Cost of Diabetic Medications of Patients Attending Selected Public Sector Diabetic Clinics in Sri Lanka

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Abstract

Background: Diabetes Mellitus (DM) is a global epidemic. Globally the number of diabetic complications is increasing. Therefore, it is important to find the reasons for the escalating number of diabetic complications and associated mortality. The main objective of this study was to evaluate patients' knowledge and level of adherence to treatments, the cost associated with diabetic medications and the level of glycemic control. **Methods:** This study was a pretested, structured questionnaire-based cross-sectional survey with retrospective data collection from participants' clinic books. The questionnaire was developed to obtain data on socio-demographic variables; patients' knowledge on disease and medicines; patients' dietary control and adherence to the medications and non-medicinal recommendations. **Results:** Patients (n = 207) attending the diabetes clinics of two teaching- and two base-hospitals in Sri Lanka were taken as the sample. The majority of the participants demonstrated moderate knowledge (63%) on DM. Of the participants, 46% had moderate adherence to pharmacological and nonpharmacological recommendations of healthcare providers and 55% showed good glycemic control. The monthly average drug cost for a DM patient was 270.10 Sri Lankan Rupes (LKR). There was a significant association between participants' knowledge and level of adherence. However, there were no significant associations between participants' knowledge and glycemic control, or patients' adherence and glycemic control. There was a significant knowledge gap between the patients attending Teaching- and Base-hospitals. **Conclusion:** The majority of the diabetic patients had either moderate or above knowledge and adherence, but their glycemic control was sub-optimal and associated with factors other than patients' knowledge and adherence.

Keywords: Diabetes Mellitus; Knowledge; Adherence; Glycemic control

Introduction

Diabetes Mellitus (DM) is a chronic disease condition that occurs due to the inability of the body to produce any or adequate amounts of insulin or to use insulin effectively which leads to hyperglycemia. There are three types of DM, Type 1 DM (insulin dependent DM (IDDM), Type 2 DM (non-insulin dependent DM (NIDDM) and gestational DM (GDM). Treatments for diabetes vary depending on the type. Type 1 DM is treated with insulin. For Type 2 nonpharmacological measures/oral hypoglycemic agents and insulin are used. Nonpharmacological measures and insulin are recommended for GDM.

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DM has become a global epidemic. In 2021, 537 million 20-79 year old people have lived with diabetes and approximately 6.7 million deaths occurred due to DM or its complications (1). It has been estimated that the total number of diabetics would be 783 million by the year 2045 (1). In 2021, USD 966 billion were spent on diabetic health care (1). Most of the health care expenditure is associated with the complications of DM (2, 3). The prevalence of diabetes in Sri Lanka in 2021 was 11.3% and ranked 3rd among the South Asian countries (1).

Controlling blood glucose levels is important to prevent complications of diabetes such as cardiovascular disease, nephropathy, neuropathy, retinopathy and foot damage. Patient-related factors affecting the expected outcomes of treatment are age, gender, educational level, occupation, dietary patterns and knowledge and adherence on disease condition (4). The aim of the study was to evaluate patients' knowledge and level of adherence to medications and glycemic control. In addition, the cost associated with diabetic medications of patients attending selected diabetic clinics was also analyzed.

Materials and Methods

Study Design

This study was a pretested, structured questionnaire based cross-sectional study involving patients attending selected diabetic clinics combined with a retrospective data collection of clinic records. Patients who were attending the diabetes clinics of the teaching hospitals Kandy and Peradeniya, and the Base hospitals Warakapola and Mirigama in Sri Lanka were considered as the study population.

Ethical Approval

Ethical approval was obtained from the Ethics Review Committee, Faculty of Allied Health Sciences, University of Peradeniya, Sri Lanka (Reference number: AHS/ERC/2018/030). Approvals were obtained from the head of each institution to carry out the study. Informed consent was sorted from each participant before data collection.

Inclusion and exclusion criteria

Type 1 or Type 2 DM patients over twelve years of age, registered in the selected diabetic clinics at least three months before inclusion in the study and provided written consent for participation were included in the study. Patients who have GDM, severe medical illnesses, including physical or mental handicaps, and patients unwilling to participate were excluded.

Sampling

A total number of 207 patients; 67, 50 and 45 patients from Teaching Hospital (TH) Kandy, TH Peradeniya and each Base Hospital (BH) were conveniently included in the study. The researcher interviewed patients waiting in the clinic queues in a manner that did not disturb the doctor's visit. Before the interview started, the study's purpose and outline were explained to the participants. The list of the medications in participants' clinic records for the last three consecutive months was recorded with their glycemic values.

Data collection

The pretested and finalized questionnaire contained three major sections: 1. socio-demographic information, 2. information on patients' knowledge and awareness of the disease, symptoms, complications, and medications, and 3. information on dietary control and adherence to the medications. The glycemic control of the patients was evaluated using the laboratory report values recorded in the clinic records.

The list of medications over the past three months was obtained from the clinic records of participants to analyze the cost of medications for each patient. Medicine unit prices were obtained from

the Department of Pharmacy, TH Peradeniya. Doses and the frequencies of medicines were considered for cost calculation.

Data analysis

Statistical Package for Social Science (SPSS 25) was used for data analysis. Associations between different variables were analyzed using the Chi-Square test and *p* values below 0.05 were considered significant.

Results

Socio-demographic data

Among the participants, 42% were males, and 58% were females. Participants were included in 20-35, 36-50, 51-65, 66-80 and >81 year age groups. The majority of the participants were older than 50 years. When considering ethnicity, the majority was Sinhalese (84%). There were Tamil (9%), Muslim (6%), and Malay (1%) ethnic participants. Participants were from different educational levels. Of them, 5% had not attended school, 15% had received primary education, 27% had grade 6-9 education, 28% had General Certificate of Education (G.C.E) ordinary level (O/L), and 19 % had G.C.E. advanced level (A/L) education. Further, there were 2% diploma holders and 3% degree holders. Of the participants, 57% were not employed, 17% were employed, 24% were retired, and 2% were students.

Knowledge assessment

Knowledge of the participants was assessed using six questions related to causes, symptoms, recommendations, complications, and own antidiabetic medication. Marks were allocated (maximum 16 marks) for the answers. According to the marks obtained by the participants, they were categorized into good, moderate, or poor knowledge. The majority (63%) of the participants were in the moderate knowledge category. Further, 14 % of the participants had mentioned that they were not received health care advice from their healthcare providers. Table 1 summarises the distribution of participants' knowledge of diabetes concerning sociodemographic variables.

Adherence assessment

Adherence to treatments and non-medication recommendations was assessed using four questions. The majority (46%) of the participants had moderate adherence, whereas 35% of participants poorly adhered. Table 1 summarises the distribution of participants based on their adherence with respect to sociodemographic variables.

Glycemic control assessment

Of the participants, 55% had good glycemic control. Table 1 summarizes the distribution of participants based on their glycemic control status with respect to sociodemographic variables.

Average monthly drug cost calculation

The monthly average drug cost for a DM patient was 270.10 LKR. The average monthly cost distribution of each medicine is shown in Figure 1.

Table 1. Distribution of participants based on knowledge, adherence, and glycemic control concerning sociodemographic variables

Variable	Participants knowledge %				Participants' adherence%				Participants' glycemic control%		
	Count	Good	Moderate	Poor	Count	Good	Moderate	Poor	Count	Good	Poor
Age											
20-35 years	3	33.3%	0%	66.7%	3	33.3%	0%	66.7%	3	33.3%	66.7%
36-50 years	25	36.0%	60.0%	4.0%	25	8.0%	64.0%	28.0%	25	48.0%	52.0%
51-65 years	94	37.2%	46.8%	16.0%	94	18.1%	45.7%	36.2%	94	39.4%	60.6%
66-80 years	82	30.5%	50.0%	19.5%	82	22.0%	42.7%	35.4%	82	51.2%	48.8%
>81 years	3	33.3%	0%	66.7%	3	33.3%	33.3%	33.3%	3	66.7%	33.3%
Gender											
Male	87	40.2%	49.4%	10.3%	87	25.3%	42.5%	32.2%	87	43.7%	56.3%
Female	120	30.0%	47.5%	22.5%	120	14.2%	48.3%	37.5%	120	46.7%	53.3%
Ethnicity											
Sinhalese	174	33.9%	48.9%	17.2%	174	18.4%	45.4%	36.2%	174	45.4%	54.6%
Tamil	18	38.9%	44.4%	16.7%	18	22.2%	33.3%	44.4%	18	50.0%	50.0%
Muslim	13	30.8%	46.2%	23.1%	13	23.1%	61.5%	15.4%	13	30.8%	69.2%
Others	2	50.0%	50.0%	0%	2	0%	100%	100%	2	100%	0%
Employment status											
Retired	49	55.1%	38.8%	6.1%	49	26.5%	44.9%	28.6%	49	40.8%	59.2%
Unemployed	119	24.4%	50.4%	25.2%	119	13.4%	45.4%	41.2%	119	48.7%	51.3%
Employed	35	40.0%	54.3%	5.7%	35	28.6%	45.7%	25.7%	35	42.9%	57.1%
Student	4	25.0%	50.0%	25.0%	4	0%	75.0%	25.0%	4	25.0%	75.0%
Highest educational level											
No school	10	10.0%	50.0%	40.0%	10	30.0%	30.0%	40.0%	10	40.0%	60.0%
Grade 1-5	32	12.5%	46.9%	40.6%	32	12.5%	43.8%	43.8%	32	56.3%	43.8%
Grade 6-10	55	27.3%	52.7%	20.0%	55	14.5%	49.1%	36.4%	55	41.8%	58.2%
Up to G. C. E. O/L	58	48.3%	41.4%	10.3%	58	24.1%	36.2%	39.7%	58	34.5%	65.5%
Up to G. C. E. A/L	40	42.5%	52.5%	5.0%	40	17.0%	57.5%	25.0%	40	52.5%	47.5%
Diploma	5	40.0%	60.0%	0%	5	0%	60.0%	40.0%	5	40.0%	60.0%
Degree	6	66.7%	33.3%	0%	6	33.3%	66.7%	0%	6	83.3%	16.7%
Post Graduate	1	0%	100%	0%	1	100%	0%	0%	1	100%	0%

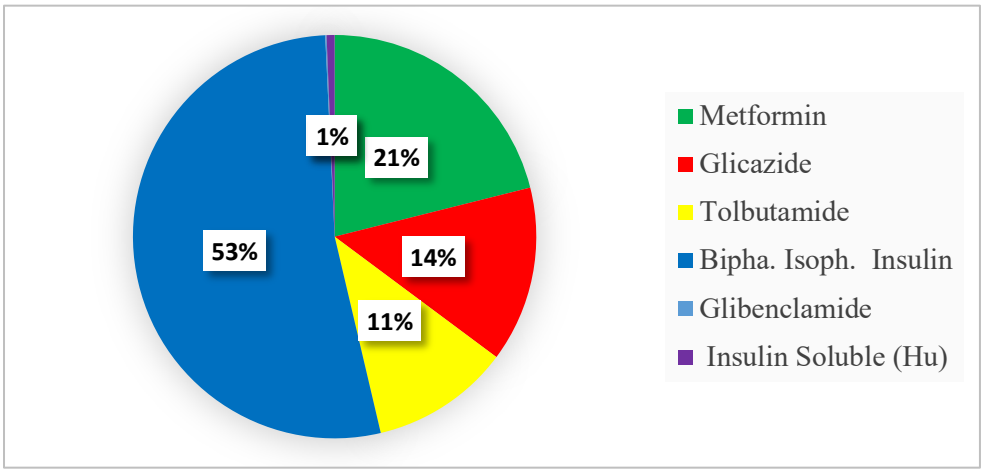

Figure 1. Distribution of average monthly cost per medicine

Association between knowledge and adherence of participants

There were significant associations between participants' knowledge and level of adherence (p = 0.028); dietary patterns and glycemic control status of patients (p<0.001); glycemic control, and average monthly costs for medicines (p = 0.041). However, no significant associations were observed between participants' knowledge and glycemic control (p = 0.374); and patients' adherence and glycemic control (p = 0.099).

Table 2 compares the level of knowledge on diabetes between the participants in Teaching and Base hospitals. Among the participants of THs (117), 29% had a good level of knowledge of diabetes, whereas the majority (61%) had a moderate level, and only 10% had a poor knowledge level. Among the BHs' participants (90), only 8% had good knowledge, and 26% had poor knowledge.

Table 2. Distribution of participants based on their knowledge level concerning their hospital setting

Hospital	Participants knowledge level on diabetese		
	Good	Moderate	Poor
Teaching Hospital	29%	61%	10%
Base Hospital	8%	66%	26%

Adherence levels between participants from selected Teaching and Base hospitals are compared in Table 3. Of the participants from THs, only 22% and 33% had good and poor levels of adherence to the medication therapies and other recommendations. Among the BH participants from BHs, only 14% had a good level of adherence, while 39% had poor adherence.

Table 3. Distribution of participants based on their adherence level with respect to their hospital setting

Hospital	Particpants adherance to medications and non medication recomendations		
	Good	Moderate	Poor
Teaching Hospital	22%	45%	33%
Base Hospital	14%	47%	39%

Table 4 compares the glycemic control status between the participants from selected Teaching and Base hospitals. Of the THs' participants 39% had good glycemic control whereas 61% were of poor glycemic control. Among the participants from BHs, 53% of participants had a good level of glycemic control, while 47% had poor glycemic control.

Table 4. Distribution of participants based on their glycemic control status concerning their hospital setting

Hospital	Particiants glycemic control status	
	Good	**Poor**
Teaching Hospital	39%	61%
Base Hospital	53%	47%

Discussion

This study was conducted in two THs and two BHs in Sri Lanka to assess patient-related factors and evaluate the outcomes related to DM. Unlike most studies in developing countries reporting patients' poor knowledge of DM, the current study shows that the majority (>83%) had moderate/above moderate knowledge. In addition, gender, age, and ethnicity had no significant association with knowledge of DM. The current findings related to knowledge of diabetes in males and females were different from other reported studies conducted in developing countries., In Bangladesh, males were found to have higher knowledge about the disease (5-7). Previous studies from developing countries and the current study revealed a significant association between level of education and knowledge of diabetes (8-10). However, there was a significant knowledge gap between participants of THs and BHs.

Non-adherence is the major cause leading to increased complications and mortality of diabetic patients. Results of the study revealed that 65% of the participants had moderate or above moderate adherence. There was a significant association between patients' knowledge and adherence ($p = 0.028$). Many previous studies in similar settings to Sri Lanka reported poor adherence, but those reported that the patients' knowledge was also poor (6, 7, 9, 10).

Diabetic dietary plans and physical activities are among the recommended non-drug management procedures for diabetics (11). The current study revealed that 65% of the participants followed diabetic dietary plans. In addition, 51% were used to engaging in physical activities on a regular basis. However, nearly 58% of the participants were non-adherent to their medicines. The results were similar to other studies (12). Healthcare instructions received from healthcare providers could have enhanced knowledge of DM in 83% of the participants. Some studies have reported that medication adherence is satisfactory when health care providers are emotionally supportive and treat patients as equal partners (13).

Most of the diabetic outpatients in the present study had poor glycemic control status (55%). The present finding is consistent with previous studies conducted in Asia and America (14,15). The current study revealed that participants' glycemic control is dependent on variables other than patients' knowledge of the disease and adherence to medication, dietary, and exercise-based recommendations. Unavailability of particular medicines in government hospitals could be one of the reasons for this poor glycemic control. The possibility of prescribing new medicines for glycemic control is a barrier in Sri Lankan government hospitals. Poor glycemic control in patients indicates a need for further studies to find the associated reasons and correct the issue. Another fact is that, though the HbA1c test is the better predictor of glycaemic control (16), in Sri Lankan government hospitals, it is performed once a year or in special circumstances only, which is hardly adequate for correct predictions.

To the best of our knowledge, this study is the first attempt to measure a DM patient's total average drug cost per month in outpatient clinics in Sri Lanka. The calculated average drug cost per month is

270.10 LKR. However, the study did not consider other associated costs and the costs for drugs other than antidiabetic agents. There was an association between glycemic control and the average monthly cost of a patient ($p = 0.041$). It reflects that the higher the cost, the treatments more effective. It could be that the patients on comparatively expensive insulin therapy, show higher glycemic control.

Conclusion

The findings of this study revealed that, even though the majority had either moderate/above moderate knowledge and adherence, their glycemic control status is sub-optimal and is associated with factors other than patients' knowledge and patients' adherence to treatment and other non-pharmacological recommendations.

Author Contributions: Conceptualization, CPTL; methodology, BDM and CPTL; validation, BDM; formal analysis, BDM; investigation, BDM; data curation, BDM; writing—original draft preparation, BDM; writing—review and editing, CPTL; visualization, CPTL; supervision, CPTL; project administration, BDM. All authors have read and agreed to the published version of the manuscript.

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Protocol

Development of an Optimal Short Course for Pharmacy Students in Sri Lanka to Enhance the Knowledge of Antimicrobial Resistance

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Abstract

Antimicrobial resistance (AMR) has been identified as one of the greatest threats to human health. Healthcare professionals play a pivotal role in patient care activities, and an understanding of antimicrobial agents and AMR is a critical component of high-quality care. Pharmacists are experts in medicines, and their education and training can directly impact the quality of services they provide to patients. Antimicrobial stewardship (AMS) competencies for pharmacy students have been developed and implemented in the developed world. However, in developing countries like Sri Lanka, there is a gap in the training of pharmacy students in AMS competencies, thereby hindering their ability to be effective in stewardship activities upon graduation.

This study presents a protocol that includes learning outcomes and content to explore a national consensus on AMS competencies that will improve knowledge about antibiotics and AMR in pharmacy students in Sri Lanka. The first draft of the proposed curriculum was developed through a literature review, informed by extensive investigations of pharmacy students' current knowledge and understanding and ensuring suitability in the Sri Lankan context. A process of liaising with academics and stakeholders in Sri Lanka and discussions with academics from developed countries (such as Australia) was used to prepare the final draft. Future consultation and implementation will also seek further input from current and recently graduated pharmacy students in Sri Lanka. The competencies developed will apply to pharmacy undergraduate programs in Sri Lankan universities and for practicing Sri Lankan pharmacists in a continuous professional development program. Once implemented, this protocol will help strengthen AMS education amongst pharmacists in Sri Lanka and ultimately benefit Sri Lankan consumers and assist other health care professionals addressing AMR.

Keywords: Pharmacy education; Health Sciences students; Antimicrobial resistance; Antimicrobial agents; Antimicrobial Stewardship

Introduction

The increasing prevalence of multi-drug resistant infections represents a major threat to global health (1). The World Health Organization (WHO) has warned that without immediate action, we will return to the pre-antibiotic era when common infections were often fatal (2). WHO implemented a global action plan on antimicrobial resistance (AMR) to overcome this challenge in 2015. Healthcare professionals' education and training is one of five key action areas in this global AMR plan (2). Education is a

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fundamental tool to combat AMR and is necessary to deliver effective and safe care (3). Education of health care students' knowledge of antibiotics is crucial (4). Various healthcare disciplines, such as medicine, dentistry, pharmacy, nursing, and veterinary science, are in a position to prescribe antibiotics and influence the use of antibiotics (5). Hence, adequate education and training is essential for these disciplines to ensure optimal use of antibiotics in hospital and community settings.

Pharmacists are essential members of the healthcare team and play an important role in medicine management and the appropriate use of medicines, including antibiotics (6). Undergraduate education provides an opportunity to prepare competent pharmacists to deliver effective professional roles in the community (7). Continuous professional education and development is another important tool for practicing pharmacists to improve their knowledge of antibiotics and AMR (8). Many developed nations have realized the importance of antimicrobial stewardship (AMS) courses for pharmacy professionals and have started to include these competencies in their undergraduate programs (5, 9). However, AMS programs for undergraduate pharmacy students in developing countries have not been widely established. In these countries, antimicrobial courses within undergraduate pharmacy programs tend to focus on basic microbiology, the classification and use of antibiotics, and therapeutic drug monitoring (10). Research shows that pharmacy students believe that robust knowledge of antimicrobials is important for their pharmacy careers and desire more education on rational antimicrobial use (11). Several studies have reported the need for enhanced antimicrobial education during undergraduate degree programs (12, 13). These studies demonstrated inadequate education about antibiotics and AMR in health care educational programs. After graduation, a professional curriculum on AMS has shown improved knowledge and attitudes toward judicious antibiotic use and collaboration with healthcare colleagues (14).

Our recent study (15) from Sri Lanka demonstrated that pharmacy education is clearly associated with an improved understanding of appropriate antibiotic use and AMR among senior undergraduate pharmacy students (15). Another recent publication by the authors compared knowledge and use of antimicrobials among pharmacy undergraduate students in Sri Lanka and Australia (16). This report identified gaps in knowledge and use of antimicrobials among Sri Lankan pharmacy students (16). These studies indicate a need to minimize these gaps in pharmacy students attending Sri Lankan universities.

This study, therefore, aims to develop a suitable curriculum for pharmacy students in Sri Lankan universities that will enhance their knowledge of antimicrobials and AMR and stewardship activities. The aim is to provide a module that can be incorporated into the undergraduate pharmacy curricula and assist future capacity building initiatives through education that will mitigate the emergence and threat of AMR. Furthermore, this curriculum aligns with the WHO competency framework for health workers' education and training on antimicrobial resistance in order to produce a standardized curriculum (17).

Methods

Module development

Strategies followed for developing the AMR curriculum for pharmacy students in Sri Lankan universities are shown in Figure 1.

The first draft of the proposed curriculum that included learning outcomes, subject topics, and content was developed through a literature search strategy. The following 'Medical Subject Headings' (Mesh) terms were used to search articles in databases such as EMBASE and MEDLINE: (Education.mp) OR (Pharmacy education.mp) OR (undergraduate professional education.mp) OR (Health professional education) AND (Antimicrobial stewardship.mp.) OR (Antibiotics.mp) OR (Antimicrobial resistance.mp) AND (Pharmacy.mp). In addition to this search strategy, the WHO's recently published AMR competency framework for healthcare professionals (17) was taken into consideration. Recent publications on AMR curriculum development and implementation for various healthcare professionals in South Asian countries were also taken into consideration.

Furthermore, the experiences of the lead researcher/academic (MHFS) regarding the local academic and healthcare systems were also considered during the preparation of the first curriculum draft. The acquired knowledge and skills while working as an academic at a Sri Lankan university was an important aspect since the researcher (MHFS) worked as a resource person to develop the B. Pharm curriculum when it was first introduced to Sri Lanka in 2005 at the University of Peradeniya. The researcher (MHFS) had also assisted in B. Pharm curriculum reviews at three different universities in Sri Lanka during 2011, 2012 and 2014.

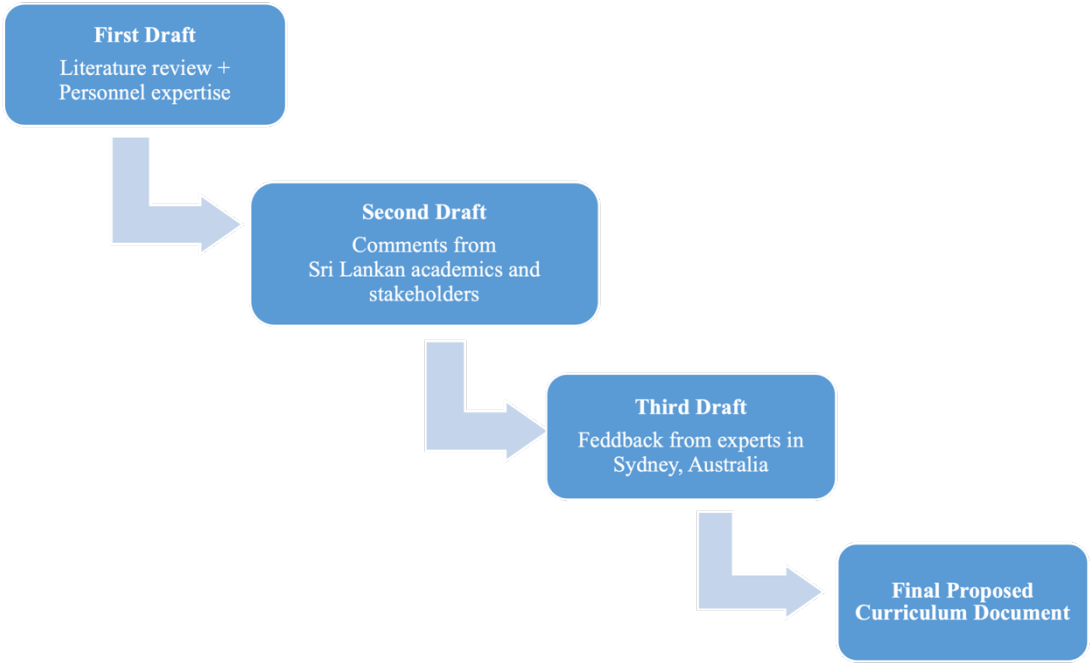

Figure 1. Strategies for the development of optimal AMR curriculum for pharmacy students in Sri Lankan universities

The development of the second draft incorporated feedback from Sri Lankan academics and stakeholders via e-mail communications. Learning outcomes and the content outlines of the first draft were discussed, and review recommendations were incorporated. For the preparation of the third draft, feedback was obtained from academic and clinical experts at the Sydney Pharmacy School and NSW Therapeutic Advisory Group. These individuals have extensive experience in the pharmacy curriculum and ample knowledge about antibiotics and AMR. The final draft was prepared by incorporating recommendations from the collective discussions and feedback from the Sri Lankan and Australian contributors.

Figure 2 demonstrates how comprehensive education and training about antibiotics and AMR for pharmacists can reduce the development and spread of AMR in the community and hospitals. This figure explains how this can be achieved through different strategies such as providing awareness of antibiotics and AMR to patients and consumers in the community. Furthermore, providing appropriate advice on antibiotic use for consumers in the community and unnecessary/inappropriate prescribing of antibiotics to prescribers in the hospital.

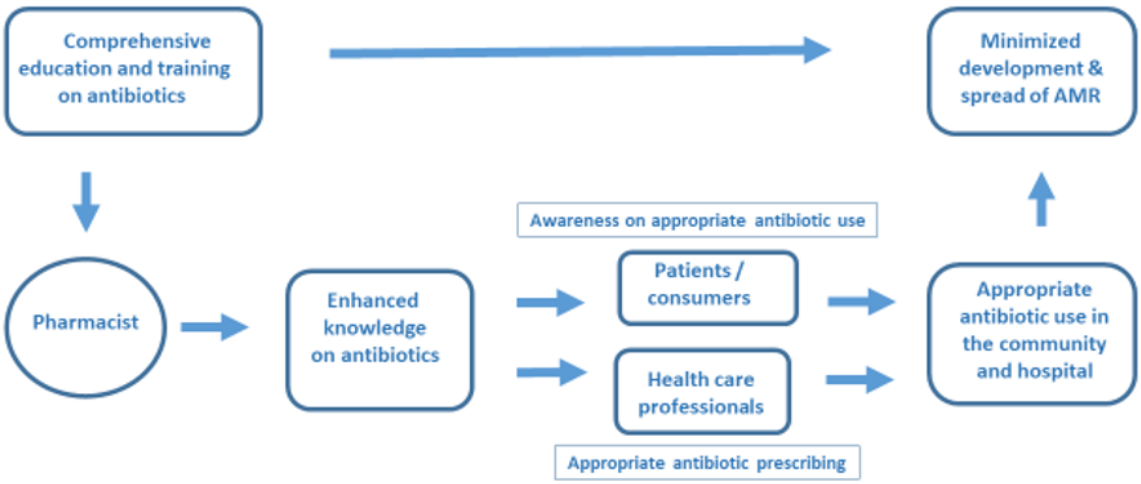

Figure 2. Comprehensive education and training on antibiotics for pharmacists to reduce the development and spread of AMR

Results

The learning outcomes and the content of this curriculum were prepared in reference to selected articles from the literature (18-21). This curriculum was prepared to fit the local context, aligning with recently published WHO guidelines on AMR education for healthcare professionals, particularly pharmacists (17). The curriculum development process (Figure 1) leads to considerable refinements of the first curriculum draft. In this curriculum, WHO guidelines are considered a general guide, and the local context incorporated is considered a special case.

Table 1 lists the main domains and learning outcomes for this newly proposed curriculum that seeks to produce competent pharmacists with a detailed understanding of the national and global challenges of AMR and the evidence-based stewardship interventions that will mitigate these challenges in hospital and community settings. The resulting curriculum covers six domains: infection and surveillance, antibiotics and antimicrobial resistance, infection and prevention control, quality use of antimicrobial agents, patient education and counseling, and interprofessional collaborative practice. Table 2 provides further details on each topic. The proposed AMR course curriculum will include topics such as antimicrobial agents, antimicrobial resistance, infectious diseases, principles of infection management, patient-centered care, and antimicrobial stewardship. A detailed content to be taught under each topic is also included in Table 2.

Discussion

Standardized curricula content that aligns with the WHO competency framework for health workers' education and training on AMR can enhance the impact of AMR and AMS education and improve suboptimal practice (17). In this project, a curriculum has been designed and proposed to improve knowledge of antibiotics and AMR among Sri Lankan pharmacy students and practicing pharmacists. This curriculum can be incorporated into all Sri Lankan universities teaching pharmacy. The competencies developed in this curriculum aim to fill the current gaps in the AMS education of the Sri Lankan pharmacy profession. The gaps in knowledge about antibiotics among pharmacy students in Sri Lankan universities have been addressed in our earlier publications (15, 16) which rationalize the need for developing a new curriculum to enhance the knowledge regarding antibiotics and AMR.

Table 1. Competency domains and proposed learning outcomes of AMR course

Domain	Proposed learning outcomes
Infection and surveillance	At the successful course completion, the students will be able to: 1. explain how microbiology testing leads to the diagnosis of infection 2. describe the data on infectious diseases at national and regional level
Antibiotics and antimicrobial resistance	At the successful course completion, the students will be able to: 1) define antimicrobial resistance (AMR) and appropriate antimicrobial use 2) describe the factors that contribute to antimicrobial resistance 3) describe the current and future potential status of AMR 4) describe at least two different mechanisms of action by which antibiotics treat infections 5) describe the challenges and impact of AMR at national and regional levels a. describe the appropriate use of antimicrobial agents in humans, food production and agriculture
Infection and prevention control	At the successful course completion, the students will be able to: 1) define the term microorganism. 2) describe different types of infectious microorganisms 3) recognize the symptoms of infection
Quality use of antimicrobial agents	At the successful course completion, the students will be able to: 1) identify the roles of the community and hospital pharmacists in facilitating rational prescribing of antimicrobial agents 2) select an appropriate antimicrobial drug (when a drug is needed). individualise dose selection and make recommendations on frequency and duration of use 3) know the importance of monitoring antimicrobial agents to inform their optimal and safe use
Patient education and counselling	At the successful course completion, the students will be able to: 1) apply patient-centered interventions such as education and medication counselling to improve judicious and appropriate antibiotic use
Inter-professional collaborative practice	At the successful course completion, the students will be able to: 1) apply multidisciplinary problem-solving approaches, including pharmacist-led interventions to address antimicrobial resistance and rational use of antimicrobial agents

These differences in the knowledge base of future pharmacists highlight the need for mapping a curriculum, which will lead to workforce capacity development. Importantly, introducing this new curriculum in Sri Lanka aligns with the workforce development goals of the International Pharmaceutical Federation (FIP) (22), which are undertaken to expand future capacity development and improve medication use. This curriculum is now proposed to be incorporated into the undergraduate pharmacy training and introduced as a continuous professional development program for practicing pharmacists. This curriculum provides a foundation for Sri Lankan pharmacy professionals to become leaders and advocates for AMS. Moreover, this curriculum will be useful for pharmacists to be involved in AMS-related medicine management activities and actively engaged in patient care.

Table 2. Topics and contents need to be included in the proposed AMR course curriculum to be implemented in Sri Lanka

Topics	Contents
Antimicrobial agents	Introduction to antimicrobial agents, the spectrum of activity, principles of empirical vs. directed antimicrobial therapy, pharmacology (including pharmacokinetics, ADME), mechanism of action and adverse effects. Significance of antimicrobial choice, dosage, duration and national medicines regulations on antibiotics, antiviral and antifungal agents
Antimicrobial resistance	Introduction to AMR, contributing factors for the development and spread of AMR, genetics and mechanisms, extent and causes, and relationship to antibiotic use. Use of antimicrobial agents in food production and agriculture and how this contributes to the spread of AMR. Local AMR patterns and their importance including AMR trends in regional and national AMR data
Infectious diseases	Standard infection prevention and control precautions, explain the link between antimicrobial stewardship and infection prevention control, explain the impact of nosocomial infections compared with community-acquired infections, use of microbiology samples and rapid point-of-care testing to identify infections
Principles of infection management	Importance of antimicrobial administration timing, determine and verify antibiotic allergies, reconcile and adjust antibiotics, report adverse drug reaction at all transitions and changes in patient's condition, importance of monitoring toxicity reliably and how to make dose adjustments
Patient centred-care	Techniques in advising patients and the prescribers on the appropriate use of antimicrobial agents, safe disposal of unused antimicrobial medicines, patient safety and medication adherence, and use of antimicrobials in compliance with formulary protocols, assessment of information and pharmaceutical products as part of good procurement practices. Techniques to use in patient discussions on why an antibiotic is not necessary and prudent antibiotic use
Antimicrobial stewardship	Definition of antimicrobial stewardship (AMS), contribution of appropriate antimicrobial use to reduction of AMR, principles of good antimicrobial prescribing and the roles of prescribers, pharmacists and nursing staff in ensuring good practice including prescription-only dispensing practices. Pharmacists' interventions to prevent health-associated infections, unnecessary prescribing of antimicrobial agents, national antimicrobial strategy and policy, AMS initiatives in developing countries and its significant outcomes

In Sri Lanka, antibiotic stewardship activities are emerging (23). Much needs to be done to improve education in this area. There is an urgent need to develop and evaluate new and improved education techniques, curriculum content, and interventions that not only increase knowledge and understanding of antibiotics and AMR but improve antibiotic use, dispensing practices, and management. Pharmacy students in Sri Lanka do not have a course that specifically focuses on AMR. Although antibiotics are discussed in several different modules, such as microbiology, pathology, pharmacology and pharmaco-therapeutics, there is limited attention given to AMR. The strength of the proposed curriculum is that it draws on evidence-based design aspects to integrate information and training in AMR delivered to the student/pharmacists in patient care centered context. One limitation of this approach to curriculum development is that there was limited consultation with current and recently graduated pharmacy students in Sri Lanka. This will be an important aspect of the implementation of the proposed curriculum.

While there is a clear need to develop standardized curricula of antibiotic stewardship in Sri Lanka for all healthcare professionals, including pharmacists, it is also important to consider the postgraduate competencies of all Sri Lankan healthcare professionals to ensure appropriate antibiotic prescribing, dispensing, and management. Postgraduate pharmacy interns should be held accountable to master and

demonstrate these essential competencies. The proposed curriculum will assist professional regulators and curriculum reform committees in Sri Lanka, given the evolution of the pharmacists' role in direct public health and patient care.

For practicing pharmacists, continuing professional education provided by hospitals, national professional pharmacy / medical societies, and public health entities should include education on antimicrobial stewardship (24). A coordinated and multifaceted educational approach, in combination with other current and future antibiotic stewardship activities, is critical to improving antibiotic use, reducing adverse events caused by antibiotics, and attenuating the development and spread of antibiotic resistance.

It is recommended that this developed curriculum for Sri Lankan pharmacists is implemented in the near future. Post-curriculum implementation, simulated client (SC) visits could be employed to explore the appropriate dispensing, advice, and counseling of antimicrobial agents at community pharmacies in Sri Lanka (25). The SC visits would support an audit and feedback model for quality improvement of pharmacists' practice. This evaluation model would further inform any enhancements that might be required to the curriculum. In addition, this curriculum could be extended to Sri Lankan allied health professionals, such as nurses, radiographers, physiotherapists, and medical laboratory scientists, strengthening their knowledge of antibiotics and AMR and enabling these professionals to practice and also reinforce critical messages and activities that will reduce the emergence of AMR in Sri Lanka.

Conclusion

This curriculum was developed to provide pharmacy students with the necessary competencies to ensure the appropriate use of antimicrobial agents and better understand the national and global challenges of AMR. This curriculum provides a clear pathway for career progression. We suggest that this curriculum be incorporated into the pharmacy academic programs in Sri Lankan universities. This would produce pharmacists with enhanced knowledge on the appropriate use of antibiotics and the harm of inappropriate use. Eventually, these competencies can be applied to their work as professional pharmacists and potential patients. Further work is needed to extend the scope of this curriculum to encompass certificate and diploma level pharmacist training.

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Sri Lankan Journal of Health Sciences

Mini Review

Effects of Human Milk Oligosaccharides on Microbiome

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Abstract

Human Milk Oligosaccharides (HMOs), the complex, unique glycans present in human milk, are essential for infants' early development and provide many health benefits to humans. Although the effects of all the individual HMOs are not fully understood, studies have revealed many health benefits of HMOs acting as prebiotics. They shape the gut microbiome, provide immune-modulatory and anti-adhesive effects to enhance innate and acquired immunity in the host and modulate the epithelial barrier. The effects of HMOs are not only limited to the infant gut microbiome but also affect the microbiome of breast milk and adults. This mini review highlights the recent findings on the effects of HMOs on the microbiome. It discusses the benefit of synthesizing and identifying the structures of individual glycans for further improvement in human health.

Keywords: Oligosaccharides; Glycans; Microbiome; Breast milk; Prebiotics

Introduction

Breast milk, the gold standard for infant nutrition, is rich in many bioactive components with complete nutrition that leads to the healthy development of the newborn. Human milk is rather a dynamic and complex nutrition source essential for the infant's growth and health that may change with some factors such as maternal diet (1), bacterial composition in milk (1), secretor and non-secretor status of the mother (2, 3, 4, 5) and environmental conditions (6). So, it is recommended by the World Health Organization (WHO) to feed the newborn exclusively with breast milk for at least six months.

Breast milk consists of lactose, fatty acids, and proteins, and among these, human milk oligosaccharides (HMOs) are considered the third most abundant macromolecular component after lactose and fat. HMOs are a complex group of glycans made out of glucose (Glc), galactose (Gal), N-acetylglucosamine (GlcNAc), fucose (Fuc), and N-acetylneuraminic acid (Neu5Ac) or sialic acid. They are mainly indigestible sugars by the infant and unique only to humans. Emerging evidence shows that more than 200 different structures of HMOs are present in human milk. More frequent feedings can ingest a high amount of HMOs into the infant, causing significant positive associations with the gut microbiome (4).

The gut microbiome is the total of microorganisms and their collective genetic materials in the gastrointestinal system. A healthy gut microbiome causes the child to be physically and mentally healthy. Certain gut microorganisms utilize HMOs in specific ways, such as a carbon source for their growth (6, 7), and HMOs play an essential role in the development of gut microbiota, acting as decoys for pathogens,

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preventing disease and infections (6), enhancing gut-barrier function and developing innate (8) and acquired immunity in infants. Now the research field is advancing to identify more functions of HMOs for the better and long-term health of infants. In infants and adults, including the mother, HMOs play a major role in giving many health benefits (9, 10). HMOs such as 2'FL and LNnT restore homeostasis in dysbiotic adults by promoting the growth of beneficial Bifidobacteria and Actinobacteria and reducing the abundance of Firmicutes and Proteobacteria (9). These HMOs promote the gut barrier function in adults strengthening the gut barrier (10). Due to these benefits and future application in therapeutic purposes and as food additives, research is continuing to identify the structures and effects of HMOs, how to synthesize them, and the therapeutic benefits of individual HMOs. The recent findings on the effects of HMOs on the microbiome of infants, alteration of microbial composition in mother's milk (11), regulation of immune function in adults (10), fight against pathogens, and important recent findings to consider in further studies related to HMOs are discussed in this mini review.

Methods

Original, recent research articles were searched using relevant keywords in PubMed and Mendeley. Next, the articles were sorted out considering the most pertinent epidemiological studies, published year from 2014 onwards, and the article type as journal articles. Then, the selected articles were examined thoroughly to gain much insight into the effects of human milk oligosaccharides on the microbiome. The relevant findings were included in the mini review.

Results

Human Milk Oligosaccharides

Human milk oligosaccharides are complex, unconjugated glycans, highly concentrated in human milk and unique only to humans. Very high HMO concentrations can be observed in the colostrum (12). HMOs are incorporated of Glucose (Glc), galactose (Gal), N-acetylglucosamine (GlcNAc), fucose (Fuc) and sialic acid (Sia), and there is evidence of more than 200 HMOs that have been identified with 3-22 units of monosaccharides (13). Since HMOs are not absorbed or metabolized in the gastrointestinal tract and enter the lower section of the gastrointestinal tract without any change (13). They act as human milk prebiotics, serve as antiadhesives, act as antimicrobials, and alter epithelial and immune cell responses (12).

Effects of HMOs on infant microbiome

HMOs are well known to support beneficial microorganisms such as *Bifidobacteriaceae,* and they are an abundant group in the gut microbiome of infants and toddlers (4, 6, 14). HMOs have strong bifidogenic effects (14), causing a long-term influence on infant and toddler health as prebiotics. They maintain the presence and activity of Bifidobacteria during the diversification of the gut microbiome following weaning. HMOs act as metabolic substrates for commensal bacteria (1). They digest complex carbohydrates and dietary fibers beneficial for human health. HMOs distinctly modulate intestinal microbiome composition and activity by promoting the growth of *Bacteroides* and Bifidobacteria (15). This group produces acetate, a major metabolite of fermentation of 2'-FL, one of the main oligosaccharides found in milk. 2'-FL and sialyl lactose increase the production of butyrate (13), propionate and lactate (10, 15), leading to many health benefits in the host.

HMOs in human colostrum also influence the human milk microbiota, mainly bifidobacteria, subsequently altering the infant gut microbiota after breastfeeding (11). It has been discovered that various microbial groups such as streptococci, staphylococci, enterococci and bifidobacteria have strong positive correlations with different HMOs (11). In infant cultures, where the effects of Sialyllactose (SL) were investigated by impaling SL to the fecal cultures, specific growth of *B.prevotella* group and *B.thetaiotaomicron* have been induced by SL and in adults, SL is reported to induce the growth of *F.prausnitizii* (15). These Bacterioides and Prevotella genera belong to the Bacteroidetes phylum, which mainly inhabits the human gut and have proven to be influenced by several HMOs.

There are significant intrinsic differences between the luminal and mucosal compartments in the microbiome compositions in the various gut regions, such as the proximal and distal colon. So, the effects of HMOs, such as 2'-FL (2'-O-fucosyllactose), show minor differences due to the variation of microbial community composition in these regions (14), and the effects are HMO specific (16). The SHIME model, a multi-compartment dynamic simulator of the human gut, 2'-FL increases *Bifidobacterium* population and increases *B.coccoides/E.rectale* in the proximal colon in adults and the distal vessel, only the *Bifidobacterium* population increases with regards to *B.coccoides/E.rectale* (10). The effects of HMOs are structure and bacterial strain-dependent as different HMOs such as 2'-FL, 3'-FL(3-fucosyllactose), 6'-SL (6-sialyllactose) and LNT2 (lacto-N-triose) affect differently in the growth patterns of various bacterial strains (16). From the mentioned HMOs, 6'-SL modulates the growth of *B.longum infantis* subspecies, and further growth is seen in co-cultures. So, HMOs selectively stimulate beneficial microbial communities' growth and metabolic activity, which means the type of HMO predicts the growth of specific types of bacteria (5). Though many studies reveal the growth improvement of microbiota, another study has discovered that pooled HMOs have the ability to inhibit bacterial growth. HMOs act as antibiofilm agents against Group B *Streptococcus* (GBS), a bacterial pathogen (17).

Fucose, one of the building blocks of HMO can be added to the HMO backbone in α 1-2- linkage, which is catalyzed by an enzyme fucosyltransferase 2 (FUT2). FUT2 active expression with high concentrations of α 1-2- fucosylated HMOs in milk can be seen in secretor mothers. Non-secretor mothers can be identified with a lack of α 1-2-fucosylated HMOs, such as 2'FL or lacto-N-fucopentaose 1 (LNFP1), having no active expression of FUT2 (12). A study has shown that the mother's secretor status does not significantly impact the infant's gut microbiota (2) and causes fewer effects on the milk microbiome (3). However, another study has discovered that the abundance of *Bifidobacterium* spp. is significantly higher in the secretor mothers' infant gut (3). Also, it has been revealed, that non-secretor mothers who are low in detectable concentrations of α-1-2-linked fucosylated HMOs in milk can have infants with increased abundance of enteropathogens and decreased abundance of *Bifidobacterium* due to the influence of gut microbial factors such as fortificant iron (2).

Fucosylated milk glycans shape the gut microbiome of infants (3). The microbiome composition of infants is lower than adults but shows a relatively high amount of Bifidobacteria (15). HMOs, like prebiotics, exert a strong bifidogenic effect in infants with the proliferation of *Bifidobacterium infantis, B.breve* and *B.bifidum* strains (6). These strains metabolize HMOs into short-chain fatty acids, giving the child many health benefits such as providing energy for the colon epithelial cells, maintaining the gut barrier, and showing immunomodulatory properties (13). Microbiota of older infants degrade HMOs efficiently, and they are more adapted for using indigestible HMOs for energy metabolism (4).

It has been proved that low Bifidobacteria levels cause gastrointestinal disorders in infants. But, some studies have shown that a high amount of *Prevotella* causes low morbidity in infants and there are no correlations between bifidobacteria and morbidity (6). In the studies that analyzed morbidity of infants, α (1-2) fucosylation of LNT (Lacto-N-tetraose) to LNFP I (lacto-N-fucopentaose I) in mother's milk has been reported to have an association with lower morbidity of a child by protecting from infections directly or indirectly (6). The *in-vitro* studies have discovered that breast-fed infants have fewer respiratory infections due to the anti-adhesive properties of HMOs (8).

Effects of HMOs on mother's milk microbiome

Besides the beneficial effects on the infant microbiome, HMOs impact the milk microbiome, and bifidobacteria utilize HMOs as their energy source (1, 11). The community and functional profile of the milk microbiome can be changed by the composition of specific HMOs by altering the maternal diet (1, 5). Research has proved that *B.longum* and some strains of *B.breve utilize* LNT and sialylated HMOs such as LST, respectively. So, individual HMOs influence the human milk microbiota in specific ways. The hypothesis has been made that HMO influence on milk microbiota causes the selective transfer of milk microbiome through breastfeeding (1, 11). So, pathogens in the mother's milk will minimally

transfer to the infant *via* breastfeeding. This happens because HMOs allow the growth of multiple *Streptococcus spp.,* which displaces *Staphylococcus aureus,* a common pathogen that causes mastitis. So, the bacterial diversity in mother's milk will be increased. Pushing away harmful pathogens and eventually reducing the pathogen population in the milk microbiome (1).

Genera such as *Lactobacillus* and *Bifidobacterium* are in lower abundance in breast milk, and *Streptococcus* spp., the dominant genus in the milk microbiome, alter the carrying capacity and grant a growth advantage in media with HMO (1). Due to the proliferation driven by HMOs in *Streptococcus aureus*, the mother can be prevented from having mastitis, which is associated with a reduction in milk microbiome diversity. The proliferation also establishes the complex commensal communities in the oral microbiome of the infant. The net effect of both these has been speculated as minimizing pathogens causing mastitis in maternal milk and the infant's mouth (1).

Effects of HMOs on the adult microbiome

HMOs provide many health benefits in adults by modulating the immune function, providing bifidogenic activity (9, 10), and modulating the gut barrier function (10). To restore and strengthen the gut homeostasis in adults, HMOs such as 2'-FL and LNnT (lacto-N-neotetraose) are proved to be safer and well-tolerated (9,10). The increase in the relative abundance of actinobacteria and bifidobacteria and the reduction in the relative abundance of Firmicutes and Proteobacteria in these adults highlight the specific modification of adult gut microbiota. The effect of giving powders of HMOs to be consumed with water as oral supplements to adults is dose-dependent (9). Adults subjected to Irritable Bowel Syndrome (IBS) or aging may develop a leaky gut or have an imbalanced microbiota. So, bacterial metabolites produced by fermenting HMOs are helpful in restoring and beneficially modulate the gut barrier function, restoring homeostasis in adults (9, 10). Supplementation of several HMOs than individual HMOs is considered more tolerated (9).

It has been reported in a study that the secretor status of a mother does not significantly influence the abundance of the sum of all pathogens in the maternal gut. But a higher abundance of *C.perfringens* can be observed in non-secretor mothers (2). It has been discovered that the mother can prevent pathogen colonization due to the HMO-driven proliferation of specific bacteria such as *Streptococcus* spp. in the human milk (1).

Effects of HMOs in enhancing immunity

Other than the effects on beneficial microorganisms, HMOs provide protection against pathogens by enhancing innate immunity. This is done by enhancing the growth of beneficial bacteria, epithelial cell maturation, and promoting epithelial barrier functioning (8, 10, 15). 2'-FL promotes the maturation of the immune system and exerts anti-inflammatory action (13). Similar to 2'-FL, each HMO shows unique effects on the pathogens such as viruses. Individual HMOs can only produce these effects. So, it has been discovered that specific HMOs reduce respiratory viral infections in human airway epithelial and peripheral blood mononuclear cells enhancing innate immunity (8). They interact directly with epithelial and immune cells to inhibit respiratory viral infections by inducing cytokines. HMOs, block pathogen binding to host cell surface glycans or receptors (8) as pathogen colonization starts by adhesion to the host cells. HMOs act as antimicrobial and antibiofilm agents against pathogens like Group B *Streptococcus* (17).

The type of HMO in mother's milk influences the overall immune status of the baby (5). It has been discovered that helper T cell (Th1) immunity can be stimulated by sulfonated and non-sulfonated HMOs (5,13). Specific HMOs or glycosyltransferase pathways change the milk composition allowing the infant to invest energy for growth by maintaining lower rates of infection and inflammation (6).

Table 1: Effects of Human Milk Oligosaccharides on Microbiome

Overall effects of HMOs on the microbiome	• Selectively support beneficial microorganisms e.g. bifidobacteria • Act as prebiotics • Act as metabolic substrates for commensal bacteria • Modulate intestinal microbiome composition and activity • Inhibit some bacterial growth • Fucosylated HMOs reduce the abundance of enteropathogens in infants
Effects of HMOs on infant microbiome	• Fucosylated glycans shape the gut microbiome • Exert strong bifidogenic effects • Provide energy for colon epithelial cells • Support gut barrier maintenance • Show immune-modulatory properties • Utilize for energy metabolism • Protection from infections directly or indirectly
Effects of HMOs on mother's milk microbiome	• Utilize as sources of energy • Alter the community and functional profile of the microbiome • Selective transfer of milk microbiome through breastfeeding • Prevention of mother from having mastitis • Provide a media to grow
Effects of HMOs on the adult microbiome	• Modulate immune function • Modulate the gut barrier function • Restore and strengthen the gut homeostasis
Effects of HMOs in enhancing immunity	• Enhance the growth of beneficial bacteria • Epithelial cell maturation • Promote epithelial barrier functioning • Promote maturation of immune system • Exert anti-inflammatory action • Individually show unique effects on pathogens • Block pathogen binding to host cell surface glycans or receptors • Act as antimicrobial and antibiofilm agents against pathogens • Stimulate helper T cell immunity in mother's milk • Maintain lower rates of infection and inflammation in infants

Discussion

HMOs play a vital role in the early development and shaping of the microbiome since it is the only nutrition supplied to the infant during the first few months of life. The highest amount of HMO is present in the colostrum, and the content gradually decreases with time in human milk (2, 3). But with the increase in daily milk intake, the HMO concentration ingested becomes relatively stable in the infant (4). The gut

microbiome dramatically influences the health of the human and maintains health throughout life by developing the immune system, host metabolism, and fermentation of the dietary indigestible glycans to short-chain fatty acids, which exert many health benefits (14). Gut microbiome composition varies largely among individuals, mainly among children (14), and one of the major factors that cause this variation is HMOs.

In this review, the effects of HMOs have been discussed briefly, considering the effects of the total HMO composition and the individual HMOs. Most studies have been conducted by analyzing maternal milk samples, feces samples, the synthesized, high purity chemicals, and using *in-vitro* models such as the Human Intestinal Microbial Ecosystem (SHIME) simulator used to study the function of HMOs on the adult microbiome (10). Fecal samples of the donors have been used to analyze the utilization of HMOs by different bacterial strains (4). In clinical studies, the convenience of the study subjects has been considered, and the samples have been collected within allocated time periods.

Most studies of individual HMOs have been done *in-vitro* (8, 15) or in animal models (13). So, with the positive results obtained, we can consider conducting more *in-vivo* studies. The effects of HMOs give hope to using different HMOs for therapeutic purposes. Some studies have revealed that supplying HMOs in adults is also an excellent strategy to promote the growth of beneficial Bifidobacteria (9). Even the maternal diet plays a vital role in changing the HMO composition in the milk (1). These will provide means to give solutions to enhance the health of the mother and the infant, preventing many infectious diseases. Some studies, which were done among a small group of subjects, provide the need for a large cohort study (11). Some studies were specific to a particular community (3). Those results can be compared with other communities in different geographical areas to gain much insight into the particular effects of HMOs and the factors that contribute to those effects.

In this review, the mother's secretor status is also considered, as this is related to the amount and type of oligosaccharides in her milk (3, 4). 2'-FL is reported to be the most abundant HMO in a secretor mother, and it is absent or minimally available in a non-secretor mother. Some studies have discovered the beneficial effects of 2'-FL supplementation (7, 14). Since some infants are not supplied with the particular HMO, and the results of the study done by Paganini D. *et al.* (2019) (2) and the study by Bai Y. *et al.* (2018) (3) were not the same for the comparison of secretor status and infant microbiome composition, the need for more studies on the HMO composition considering the secretor status of the mother arises.

Since every child cannot be breastfed for various unavoidable reasons, more attention has been given to comparing the effects of formula feeding against HMOs due to the vast amount of beneficial effects these sugars exert. HMOs are unique carbohydrates only found in human milk, and a slight change in the structure causes a significant impact on the activity of the particular HMO (16). All the structures of HMOs in milk are not identified yet. So, more studies can be conducted to find the structures of HMOs and include them in formulas to enhance the health benefits in non-breast-fed infants.

Conclusion

HMOs play a major role in developing and maintaining humans' short-term and long-term health and exert HMO structure and microbial strain-specific effects on the infant and adult microbiome. They act as prebiotics supporting beneficial bacterial growth, providing anti-pathogenic, immune-modulatory effects, and strengthening the host's gut barrier; due to the beneficial effects of HMOs, more attention has been given to using them in infants formulas and as therapeutic agents.

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Sri Lankan Journal of Health Sciences

Mini Review

Antibacterial Activity of Honey in Combination with Cinnamon

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Abstract

Emergence of antimicrobial resistance (AMR) is a major global concern today. High prevalence of multidrug resistant (MDR) bacteria cause the treatments to be less effective or ineffective and finally leads to higher rates of mortality. This situation urges the development of novel antimicrobial agents. Natural products have gained more attention as safe and cost-effective sources of candidate compounds. Honey possesses a potent antibacterial activity against a wide range of bacteria. High osmolarity, acidic pH, hydrogen peroxide production, and presence of non-peroxide compounds provide various mechanisms for honey to act against bacteria. Cinnamon also has the ability to act on a number of bacteria through destruction of cell membranes, anti-quorum sensing effect, ATPase inhibition and membrane porins inhibition. Thus, the combinations of honey and cinnamon have shown important interactions such as synergistic, additive and antagonistic effects due to the interactions among various constituents in them. Their combined action varies depending on the honey type, cinnamon plant species, cinnamon extract type, etc. Therefore, both honey and cinnamon can be considered as good candidates for developing new antimicrobial agents. Further studies are required to isolate and identify bioactive compounds and to clarify their exact mechanisms of action, antibacterial spectrum, and toxicities.

Keywords: Antimicrobial resistance (AMR); Antibacterial activity; Honey; Cinnamon; Synergism

Introduction

Over the last few decades, the whole world has been experiencing an antimicrobial resistance (AMR) crisis due to the persistent, irrational use and the slow discovery of novel antimicrobial agents. The evolution and high prevalence of multi-drug resistant (MDR) bacteria cause the antibiotic treatments to be less effective or ineffective against once treatable infections. To address these issues, researchers are making more efforts to develop new antibiotics. In this context, natural products from plants or animals have become major candidates for developing cost-effective, less toxic and efficacious antibiotics than synthetic compounds (1).

Honey is the natural sweetening agent produced by honey bees (genus *Apis*) using flower nectar, secretions from living parts of the plants or excretions from plant sap sucking insects, such as aphides (2). It is a complex mixture of sugars, mainly fructose and glucose, various amino acids, organic acids, lipids, vitamins, lactones, minerals, enzymes, phenolic compounds, flavonoids, etc. (3). Honey has a higher percentage (70-75%) of sugars. The chemical composition and physical properties of honey show considerable variations depending on botanical or floral sources, soil characteristics, seasonal factors, climatic conditions, bee species, honey maturity, extraction process and storage conditions (3). Honey has been proven to have important biological properties such as antimicrobial, antioxidant and anti-

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inflammatory activities (4). Because of that, honey is a popular natural compound used in traditional home remedies and Ayurvedic medicinal system, and it has a long history of being used in the treatment of various infections (5). In previous studies, honey has shown both bactericidal and bacteriostatic properties depending on the concentration of honey used and the type of the bacteria.

Cinnamon is a spice obtained from plants belonging to the genus *Cinnamomum*. Bark, leaves, fruits, flowers, and roots of the cinnamon plants are used to obtain essential oils that are useful in medicine, food and cosmetic industries. The chemical composition of essential oils varies significantly depending on the extraction method, cultivation conditions, geographical origin, growing seasons, plant maturity and the parts of the plant used to extract essential oils. Thus, their pharmacological effects may also vary accordingly (6). Cinnamon extracts contain alkaloids, flavonoids, saponins, tannins, terpenoids and glycosides in varying concentrations (7). *C. zeylanicum* bark essential oil contains 60-80% of cinnamaldehyde as the major constituent. It also contains 8-10% cinnamyl acetate and other compounds such as eugenol, linalool, benzyl benzoate and beta-caryophyllene. Eugenol is the major constituent (70-75%) in cinnamon leaves essential oil. Other than that, cymene, cinnamaldehyde, linalool, cinnamyl acetate, β- caryophyllene, and benzyl benzoate are also present in oil extracted from cinnamon leaves. Camphor is abundant in root essential oil. In addition to these volatile compounds, cinnamon also contains non-volatile compounds such as proanthocyanidins and catechins (6, 8, 9). Cinnamon has been used as a herbal remedy to treat indigestion, sore throat, diarrhea, gastric ulceration in *Helicobacter pylori* infections, toothaches, etc., and as a food spice or a natural preservative due to its antimicrobial properties (9).

Certain strategies have been suggested to overcome AMR, such as combinatory therapies and newer drug rotation (10). In order to use drug rotation, there should be higher availability of new antibiotics. Thus, combinatory therapies have received more attention as the synergistic or additive interactions of two or more combined antimicrobial agents may reduce the toxicity associated with higher doses of single agents because lower doses of antimicrobial agents may reduce dose-related adverse effects. Furthermore, they may reduce the emergence of resistance by acting on the bacteria through various mechanisms of action and thereby provide a broad spectrum of activity. This review is intended to provide an overview of the antibacterial effects of honey, cinnamon, and their combination.

Antibacterial activity of honey

Long ago, honey has been used to treat ulcers, wounds, burns, gastrointestinal infections, and infectious skin conditions. Also, it was an effective food preservative due to its profound antimicrobial activity (11). Recently, a number of studies have suggested several mechanisms for the antibacterial activity of honey. The high osmotic nature of honey contributes mainly to its antibacterial activity due to the presence of high sugar and low water content (5, 12). High osmotic pressure inhibits bacterial growth by drawing water out of bacterial cells. The acidic pH (range of 3.2 - 4.5) of honey contributes to its antibacterial activity because most bacteria require an optimum range of pH 6.5 - 7.5 to grow. The presence of various organic acids, especially gluconic acid, causes honey to be acidic (5).

Hydrogen peroxide (H_2O_2) is produced when the glucose oxidase enzyme in honey acts on glucose. Due to its low pH, the enzyme is inactive in undiluted honey and gets activated when it is diluted. H_2O_2 can oxidise various cellular components that are essential for bacterial growth such as cell membrane, proteins, and DNA (11). The presence of compounds such as phenolic acids, mainly the gallic acid, flavonoids such as pinocembrin and rutin, methylglyoxal (MGO), lysozyme, antibacterial peptides and other volatiles are also have a significant relationship with the antibacterial activity of honey (11, 12).

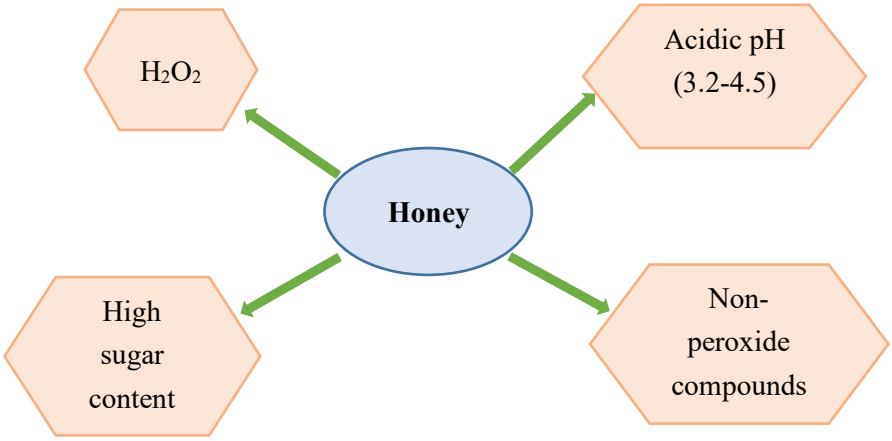

Figure 1. Characteristics of honey that support its antibacterial activity

A study conducted in 2018, has examined the antibacterial activity of 21 types of honey collected from Mount Olympus in Greece, which has high plant biodiversity, against methicillin-resistant *Staphylococcus aureus* (MRSA) and carbapenem-resistant *Pseudomonas aeruginosa.* The activities have been compared with Manuka honey, a famous traditional medicine. According to their results, all the tested honey types have shown antibacterial activity against both organisms, and *P. aeruginosa* was less susceptible than *S. aureus.* The minimum inhibitory concentration (MIC) of all the tested honey samples was in the range of 3.125-12.5% (v/v) against MRSA while MIC of Manuka honey was 6.25% (v/v). MIC against *P. aeruginosa* has been in the range of 6.25-12.5% (v/v) while, for Manuka honey, it was 12.5% (v/v). Furthermore, researchers have proven the importance of H_2O_2 and proteinaceous compounds on the antibacterial activity of honey by treating it with catalase and proteinase-K (13).

In another study, four types of honey from Ethiopia demonstrated antibacterial effects against ten clinical isolates of MRSA. Methicillin resistance has been established based on its resistance to oxacillin and cefoxitin. The antibiotics, ciprofloxacin, gentamicin, tetracycline, co-trimoxazole, chlor- amphenicol, amikacin, clindamycin, erythromycin, and vancomycin have been used to determine the antimicrobial sensitivity of MRSA, and the study has found that 80% of MRSA were resistant to tetracycline while 40% were resistant to co-trimoxazole and 30% were resistant to erythromycin. However, MRSA has shown high sensitivity to vancomycin, amikacin, ciprofloxacin, and gentamicin. From the four tested honey samples, one potent honey type, which has a MIC ranging from 9.38-37.5% (v/v) against MRSA has been found (14).

Malaysian honeys, especially "*Tualang*" honey (a wild polyfloral honey produced by *Apis dosarta*), have exhibited a great antibacterial activity against *Escherichia coli, S. aureus, P. aeruginosa* and spore forming *Bacillus cereus.* Gram-positive bacteria have shown higher sensitivity than Gram-negative bacteria. The antibacterial effect of Malaysian honey is mainly due to the non-peroxide components (15). Manuka honey is derived from the nectar of *Leptospermum scoparium* bush, which is indigenous to New Zealand and Australia (16). It is available in the market as a therapeutic honey to treat ulcers, burns and wounds. A number of studies have confirmed its excellent antibacterial activity against a range of Gram-positive and Gram-negative bacteria. Non-peroxide compounds in Manuka honey have a large contribution to its antibacterial activity (17). MGO is the most abundant non-peroxide compound present in Manuka honey, and hence, the Manuka honey grading system, the Unique Manuka Factor (UMF) reflects the MGO concentration in Manuka honey (18). MGO has demonstrated bacterial cell lysis inhibits flagellation and disrupts bacterial cell division. Manuka honey has been identified to be effective against *Alcaligenes faecalis, Citrobacter freundii, E. coli, Enterobacter aerogenes, Klebsiella pneumoniae, Mycobacterium phlei, Salmonella california, Salmonella enteritidis, Salmonella typhimurium, Shigella sonnei, S. aureus, Staphylococcus epidermidis* and MRSA (16, 17).

A study performed to compare the antibacterial activities of four honeys from New Zealand, Cuba, and Kenya has shown that all the honey types were active against the tested 51 clinical isolates of bacteria (34 Gram-positive and 17 Gram-negative). In contrast, *Melipona beecheii* (a stingless bee) honey obtained from Cuba has shown the greatest inhibitory activity due to its high acidity. Moreover, all the honey types have had the ability to inhibit biofilm formation and reduce formed biomass. Cellular structural changes have also been observed in bacteria treated with *M. beecheii* honey (19).

Sri Lankan honey has also been reported to have antibacterial potential against S. *aureus* and *E. coli.* Fourteen honey samples of *Apis cerana* and *Apis dosarta* collected from different areas of the country have shown an increasing zone of inhibition, and hence, an increasing antibacterial activity with honey concentration (20). Another study performed in Sri Lanka has confirmed that those honey samples have a potent antibacterial effect against S. *aureus* and *E. coli* with MIC values ranging from 0.125-0.25 g/mL (21). Sri Lankan honey has been reported to have a higher antibacterial effect against S. *aureus* and a similar antibacterial effect against *E. coli*, compared to Manuka Honey (22). Furthermore, twelve Sri Lankan honey samples have exhibited significant antibacterial activity against Gram-positive and Gram-negative bacteria isolated from chronic wounds (23). Nevertheless, honey has shown a greater healing effect and a significant reduction of *P. aeruginosa* count, in comparison to silver sulfadiazine when used on burn wounds of rats (24).

Antibacterial activity of cinnamon

According to previous research, trans-cinnamaldehyde is the major constituent responsible for its antimicrobial activity (8, 25). The most predominant antibacterial mechanism of cinnamon is the alteration of the cell membrane and its lipid profile. Destruction of cell membrane leads to leakage of electrolytes, proteins and nucleic acids that are essential for cell growth (26, 27). Gram-positive bacteria are more susceptible to this mechanism than Gram- negative bacteria (28). Constituents of cinnamon such as trans-cinnamaldehyde have the ability to inhibit the enzyme ATPase and thereby suppress the cellular metabolic activities that require energy (29). The cinnamon essential oil has also proven its anti-quorum sensing effect. Quorum sensing is known as an intercellular communication system based on the secretion and detection of extracellular signal molecules. By suppressing this system, cinnamon can reduce the mobility and biofilm formation of bacteria (30, 31). Trans-cinnamaldehyde also inhibits the membrane porins through reducing the gene expression of porin proteins and amino acid transporters to inhibit active transport across the bacterial cell membrane and cell division (31, 32).

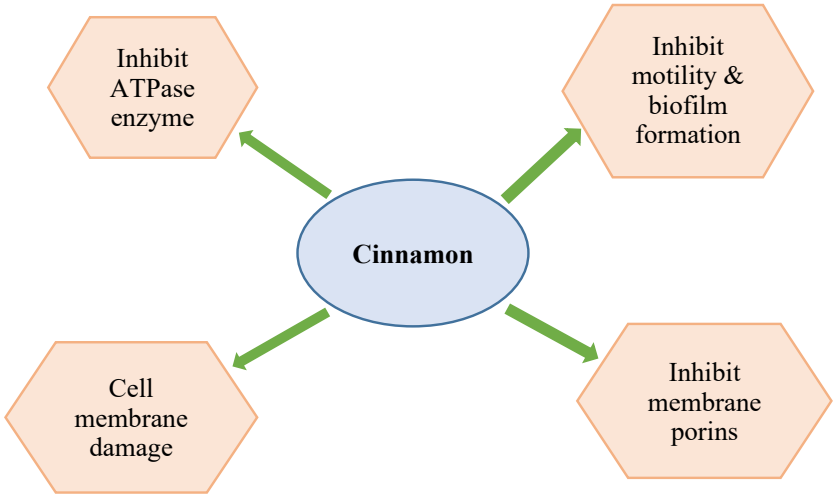

Figure 2. Mechanisms of action of cinnamon on bacterial cells

A study has revealed that cinnamon essential oil has a potent inhibitory activity on *E. coli* and *S. aureus* while, *S. aureus* was more susceptible. Researchers have confirmed a leakage of intracellular components of bacteria (33). Nine clinical samples of *Streptococcus mitis, S. sanguinis, S. salivarius, S. pluranimalium, S. pneumoniae, S. alactolyticus, Kocuria rosea, K. kristinae,* and *Spingomonas paucimolis* obtained from oral cavities have shown a significant sensitivity to *Cinnamomum brumannii* bark ethanol extract at all the concentrations tested (6.25%, 12.5% and 25%). A mouthwash prepared using the above extract has reduced the oral bacterial populations (CFU/ml) especially, *Streptococcus* growth in patients (34).

C. zeylanicum bark essential oil has also shown strong antibacterial activity against *S. aureus, Listeria innocua, Bacillus cereus, P. aeruginosa, E. coli* and *Salmonella typhi*. Gram-positive bacteria were reported as more susceptible to essential oil than Gram-negative bacteria (6). Moreover, *C. zeylanicum* bark essential oil has been reported to have an antibacterial effect against following extensively drug-resistant bacteria. This study performed using MRSA, vancomycin-resistant *Enterococcus faecium, Acinetobacter baumannii, P. aeruginosa,* and *E. coli* has shown that all the tested bacterial isolates were susceptible to essential oil. According to the study results, Gram-positive cocci were more sensitive than Gram-negative rods (35). Another study conducted in India has compared the antibacterial activity of commercially available cinnamon essential oil with methanol, chloroform and aqueous extracts of *Cinnamomum verum* bark and leaves against urinary tract infecting bacteria (*E.coli*, *S. aureus, Klebsiella pneumoniae, P. aeruginosa, Proteus mirabilis*) and *Aspergillus niger.* Results have shown that the bacteria were sensitive to cinnamon in the order of cinnamon oil > cinnamon chloroform extract > cinnamon methanol extract > cinnamon aqueous extract, when the inhibitory effects were assessed using a growth curve of bacteria. Moreover, cinnamon oil has shown a similar or larger inhibitory zone compared to streptomycin. Cinnamon has affected all the phases, while log phase prolongation is the most prominent effect. The growth of *A. niger* has been inhibited by cinnamon oil (7).

Ethanol extract from *C. zeylanicum* bark has shown a maximum antibacterial effect against *Listeria monocytogenes, S. aureus, E. coli, Enterococcus faecalis, Klebsiella pneumoniae, Enterobacter cloacae* and *Acinetobacter baumannii* over twelve other plant extracts such as lavender (*Lavandula officinalis*), clove (*Eugenia caryophylata*), thyme (*Thymus serpyllum*), rosemary (*Rosmarinus officinalis*) etc. Gram-positive bacteria have been identified as more susceptible (36). A study comparing *C. zeylanicum* and *C. cassia* bark oil against *Bacillus subtillis, Klebsiella pneumonia, P. aeruginosa, S. aureus,* and *E. coli* has shown that *C. cassia* oil has a more potent antibacterial activity (37). Studies have proven that *C. zeylanicum* essential oils have strong anti-*Helicobacter pylori* effects (38). Interestingly, cinnamon fruit extracts (benzene, ethyl acetate, methanol and water) have also shown significant antibacterial effects against *Bacillus cereus, B. coagulans, B. subtilis, E. coli, S. aureus* and *P. aeruginosa* (39).

Surprisingly, the cinnamon essential oil has shown a strong cytotoxic activity too (40, 41). Therefore, some studies suggest that the potent antibacterial activity of cinnamon is partly due to its toxic effects. A study conducted in 2007 has demonstrated that the essential oil obtained from *C. zeylanicum* is toxic to Vero cell line at concentrations above 0.00005 ml/ml (40). Thus, MIC should be lower than the minimum toxic concentration in order to use it in humans.

Antibacterial activity of honey and cinnamon combination

Both honey and cinnamon have been reported to have potent antibacterial effects. Thus, several studies have been performed to investigate the combined effect of honey and cinnamon on bacteria to check whether the combination of them would have greater effects than the individual products. Interactions between two antimicrobial agents can be classified as synergistic, additive or antagonistic (10, 42). Synergy can be explained as an interaction between two or more agents, that produces an effect greater than the sum of their individual effects. It is the most effective and most important interaction. Additivity occurs when the combined effect of the two agents is equal to the sum of the effects of each

agent. In antagonism, one agent suppresses or inhibits the action of the other. It reduces the effect of each agent (10).

A study conducted in 2017 has shown a synergistic antibacterial activity of sterilized and pasteurized Iranian honey with ethanol extract of cinnamon bark against *Streptococcus mutans* bacteria. The combination has been suggested for use in dental caries prevention and to simplify therapy against *S. mutans* (43). The synergistic activity of honey with both ethanol and aqueous extracts of *C. zeylanicum* bark has been proven against multi-drug resistant isolates of *Pseudomonas aeruginosa* obtained from burn wounds (44). Moreover, Indonesian raw honey and cinnamon bark ethanol extract have shown an additive effect against acne causing bacteria, *Propionibacterium acnes* and *Staphylococcus epidermidis* (1).

In another study, the antibacterial effect of the combination (honey with cinnamon bark ethanol and methanol extracts) has displayed an increased activity against *P. aeruginosa.* Conversely, the combinations have a reduced inhibitory effect against *S. epidermidis* and *S. aureus* (45). A similar study has reported that the addition of cinnamon to honey has reduced the antibacterial effect of honey against *S. aureus, S. epidermidis, P. aeruginosa,* and *E. coli* (46).

Conclusion

Both honey and cinnamon have displayed potent antibacterial activities against a wide range of bacteria. Combinations of honey and cinnamon have synergistic, additive, and antagonistic effects due to the interactions among their constituents. Some constituents may enhance the activity of others or provide several mechanisms for an antibacterial activity to show synergy. Meanwhile, some constituents may suppress or dilute the effects of another compound to show antagonism. However, they will be good candidates for developing new antimicrobial agents to overcome AMR. Therefore, further studies are required to isolate and identify bioactive compounds and clarify their exact mechanisms of action, antibacterial spectrum and toxicities when used alone and in combination.

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Sri Lankan Journal of Health Sciences

Mini Review

Molecular Farming: Implication for Future Pharmaceutical Products

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Abstract

The goal of molecular farming is to produce large amounts of active and secure pharmaceutical proteins at a low cost. Nowadays, gene transfer methods in plants have advanced significantly because of scientific advances in the field of biotechnology. Compared to other microbial and animal expression systems, these transgenic plants have several advantages in terms of ease of production, low cost, safety, and so on for producing pharmaceutical biomolecules. So far, many valuable pharmaceutical proteins and antibodies have been produced using this method, which has significantly aided patient treatment, particularly in developing countries where the production and preservation costs of such medicines are prohibitively expensive. However, there are some disadvantages, such as acceptance by the public, transgene escape and biosecurity, and so on, however, it is hoped that with the efforts of researchers, molecular farming will achieve great success in the near future. This mini-review highlights the history of molecular farming, plant transformation strategies, classes of protein within molecular farming, products in the market, products nearing commercialization, advantages of using transgenic plants as a bioreactor, major barriers to broader market penetration and strategies to overcome them, biosafety and challenges in the production of proteins and future prospects.

Keywords: Pharmaceuticals; Recombinant protein; Edible vaccine; Monoclonal antibody; Transformation

Introduction

Plant molecular farming (PMF) is a simple and cost-effective method (1) that involves genetically modifying agricultural products to produce high value recombinant proteins and chemicals for commercial and pharmaceutical purposes. The vast majority of developing countries are unable to afford the high costs of medical treatments resulting from the existing methods. Therefore, not only new drugs but also less expensive versions of existing drugs must be developed using low cost methods (2). Molecular Pharming represents an unprecedented opportunity to produce low-cost modern medicines and make them available on a global scale. The most promising area is infectious disease prevention, particularly in developing countries where access to medicines and vaccines has historically been limited (3).

Typically, molecular farming technology in plants has greatly concentrated on the production of pharmaceutical proteins; however, plants can also be used to produce food supplements, biopolymers, industrial enzymes, and proteins in research (avidin, glucuronidase, etc.) (4). To date, five common platforms, including mammalian cells, bacteria, yeasts, insect cells and plant cells, have been widely used

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to produce recombinant proteins (5). The ability of plants to express various proteins has now been demonstrated. However, only a few products have made it to the market due to some concerns, such as transgene amplification and diffusion, recombinant protein toxicity in the environment, food chain contamination, and subsequent processing costs (6, 7).

Academic laboratories have been critical in elucidating much of the science underlying potential products and will continue to be so they will also need to emphasize downstream process development research as the industry grows. This will supplement their fundamental work on protein expression and provide the foundational knowledge needed to fuel the sector (7). Plant molecular pharming holds significant promise for increasing global access to medicines. The time has come to accelerate the development of traditional products and new concepts (8). Original research articles were searched using relevant keywords in PubMed and Google Scholar. Then the articles were screened and 25 articles were selected to include in the review. The selected articles were reviewed, and relevant findings were incorporated into the mini review.

History of molecular farming

Plant viruses were discovered in the early 1980s. Fritz Kreuzaler and his colleagues confirmed the presence of an assembled full-size antibody after injecting cDNAs into the nuclei of *Acetabularia mediterranea,* an algal species with large cells used as a model organism in cell biology. To confirm the preselection, an anti-idiotype antibody that binds specifically to the assembled antibody was used (4).

The first recombinant pharmaceutical proteins were extracted from plants (human growth hormones), and the first recombinant antibodies were generated from transgenic plants in 1986 and 1989, respectively (6). The first molecular farming products, which were commercialized more than 20 years ago, were avidin and β-glucuronidase produced in transgenic maize plants for industrial use in 1997 (4). Since then, many diagnostic and technical proteins have been produced in plants, and some companies (e.g., Leaf Expression Systems, Agrenvec, and Diamante) have added this protein category to their portfolios (4).

Plant transformation strategies

Currently, there are two general methods for producing protein from plants: 1) Stable or permanent expression systems, and 2) Temporary or transient expression systems.

1. Stable or permanent expression system

Stable transformation entails the incorporation of a foreign gene or genes into the plant's genome. This can be accomplished in dicotyledonous plants through agrobacterium-mediated transformation or in monocots through biolistic delivery (gene-gun) methods. These transformations result in the heritable expression of a stable recombinant protein from generation to generation, making it suitable for long-term recombinant protein production (2). Stable/permanent expression systems can be further grouped as follows:

a) Stable nuclear transformation

To date, the nuclear transformation of a crop species is the most common method of producing protein from plants and has produced all the products available in the market. This system necessitates a method for transferring foreign genes into plant cells, typically via *Agrobacterium tumefaciens* or particle bombardment, in which the genes are taken up and stably incorporated into the host nuclear genome (5, 7). There are several advantages with this method. When carried out on a crop species like grains, the protein product is generally accumulated into the seeds. Then, they are harvested in dry form and stored until processing is completed. Also, this approach can be used for large areas of land at the lowest possible cost. Because crops like rice and corn are grown worldwide, the products have the potential to be produced close to the target markets (6). However, it takes time to develop stably transformed plants,

which can take months or years depending on the plant type used for recombinant protein expression (2). The other disadvantage of this method is that some grains, such as corn have the potential to cross with native species or food crops. Still, some technologies can be used to prevent outcrossing, such as mechanical detasseling or genetically based male sterility. Because of higher manual labor requirements, lower yields, and less effective genetics, such technology generally reduces the system's cost advantage (7).

(b) Stable plastid transformation

A plastid transformation system was first described using the tobacco plant (9). This system is based on the insertion of exogenous DNA into specific chloroplast genome sites via homologous recombination (10). Plastid transformation is a superior solution to nuclear transformation because it has numerous advantages, such as preventing transgene escape through amphimixis. Plastids are inherited through the maternal tissue in most species and the absence of chloroplasts in most species of pollen reduces environmental concerns (7). This system's disadvantage is that protein stability will change over time in any fresh tissue molecular farming system, even when refrigerated. Following harvest, extraction and purification must take place at precise times. Tobacco is a highly regulated crop that is not edible. This system does not appear to be capable of producing large quantities of products or edible vaccines (7). The researchers have already extracted a human pharmaceutical protein, amounting to more than 3% to 6% of the total soluble proteins in tobacco chloroplasts. Recently, a very high level (70 % of an entire soluble protein) was reported for a protein antibiotic with the chloroplast system, which was the highest concentration of recombinant proteins until today (11).

(c) Plant cell suspension culture

In-plant cell suspension culture, cell walls are removed, and genes are transferred to the obtained protoplasts and grown in suspension culture (6). In this system, transgenic plants containing a gene coding for the target protein are grown hydroponically in such a way that the desired product is released into the hydroponic medium as part of the root exudate (7). This method has been utilized as an alternative source to produce high-value bioactive compounds in some plant species (12). Plant cell suspension cultures have several advantages that make them suitable for recombinant protein production. They can be grown under aseptic conditions using traditional fermentation technology and are easy to scale up for manufacturing. The regulatory requirements are similar to those established for well-characterized production systems based on microbial and mammalian cells (13). Purification of the desired product is much easier because no tissue disruption is required, and the amount of contaminating proteins is low (7). Therefore, the purification system and its downstream processing are less expensive and easier to implement.

Furthermore, the use of suspension culture can reduce heterogeneity in proteins and sugar (N-glycans) in terms of cell type and size uniformity. Additionally, as a fast system, no transgenic plants are required. However, cell lines can be produced after a few months (6). The main disadvantage of this system is the lack of ability to produce large (kg) quantities of any protein. Further, this method is relatively costly to operate due to the use of greenhouse/hydroponic facilities (7). Plant cell suspension cultures will almost certainly become the preferred choice among plant-based systems to produce high value recombinant proteins soon because they combine the benefits of all other systems (14).

2. Temporary or transient expression systems

Transgenic plants are commonly used to obtain recombinant proteins or to identify protein localization. However, it takes a long time to create transgenic plants, and the yield of the expressed protein is relatively low. Transient expression systems, on the other hand, allow of rapid and high-level expression of recombinant proteins (15). This is because it can be accomplished through agroinfiltration using agrobacteria, viral vectors, or biolistics. During transient expression, the foreign genetic material does not integrate into the plant's genome (2). Before proceeding to the time consuming and costly stable

transformation, transient expression can be used in pilot experiments. Protein expression problems can be identified and corrected, increasing the likelihood of producing the desired protein via stable transformation. Agroinfiltration allows several genes to be expressed simultaneously, which can aid in research (2). This method is not suitable for any protein that needs to be consumed in large quantities. The product must be processed immediately because storage causes plant tissue degradation (7).

Classes of proteins within molecular farming

Proteins currently produced in plants for molecular farming purposes fall into four categories: 1) parental therapeutics and pharmaceutical intermediaries, 2) industrial proteins (e.g., enzymes), 3) monoclonal antibodies (MAbs), and 4) antigens for edible vaccines.

1. Parental therapeutics and pharmaceutical intermediates

This category includes all proteins that are directly used as pharmaceuticals and used in the production of pharmaceuticals. The list of such proteins is long and growing, and it includes products such as thrombin and collagen (both therapeutics) and trypsin and aprotinin (intermediates). In practice, only high-value proteins will be considered candidates for molecular farming.

2. Industrial protein- enzymes

This category includes hydrolases, which include glycosidases and proteases. Laccase, a fungal enzyme used in fiber bleaching and bio-glue a wood product, belongs to a distinct class of industrial enzymes. Enzymes involved in biomass conversion to produce ethanol are potential candidates for molecular farming. All these products are usually distinguished because they are used in large quantities and thus must be produced at a low cost (16).

3. Monoclonal antibody

This category includes all antibody forms (IgA, IgG, IgM, secretory IgA, and so on) and antibody fragments (Fv). Plants can produce both glycosylated and non-glycosylated forms of them. These plant-derived MAbs (plantibodies) have the potential to alleviate the severe production bottleneck that currently exists as dozens of new MAb products compete for market share.

4. Antigen for edible vaccine

Plants can produce specific protein antigens that, when consumed by an animal or a human, cause a humoral immune response. These edible vaccines were used in protection studies, they demonstrated good efficacy. In some cases, the edible vaccine provided better protection than the commercially available vaccine (17).

Products in the market

Avidin

Avidin is a glycoprotein found in the egg whites of birds, reptiles, and amphibians. It is primarily used as a diagnostic reagent (4). The protein comprises four identical subunits, each of which is 128 amino acids long. Avidin is typically obtained in commercial quantities from chicken eggs white. However, the finished product is relatively expensive due to the high cost of keeping live animals. The production of chicken egg white avidin is done using transgenic corn, which uses an avidin gene with an optimized sequence for expression in corn. The resulting avidin had properties nearly identical to those of avidin derived from chicken egg white (7).

Beta-glucuronidase

Beta-glucuronidase (GUS) is a homotetrameric hydrolase that cleaves linked terminal glucuronic acids in monosaccharides and oligosaccharides as well as in phenols. GUS is a popular visual marker in

transgenic plant research. It was first reported to be commercially produced in transgenic corn, where its properties were compared to GUS extracted from *Escherichia coli* (7).

Trypsin

A more recent introduction, maize-derived trypsin, has significant market potential. Trypsin is a protease used in various commercial applications, including the purification of some biopharmaceuticals. The availability of bovine trypsin derived from maize contributes to the growing market for animal-free reagents. Pharmaceutical companies involve in this market desire to eliminate animal-sourced materials and reduce concerns about product contamination by mammalian viruses and prions (7).

Products nearing commercialization

Many companies have started to produce molecular farming products over the last several years. Several of these proteins are normally derived from animal organs. Due to the possibility of animal pathogens being carried along with these proteins, there is a need for low-cost alternatives (7).

Several Pharmaceutical Derived Protein (PDP) products for the treatment of human diseases, including recombinant gastric lipase for the treatment of cystic fibrosis and antibodies for the prevention of dental caries and the treatment of non-Hodgkin's lymphoma, and Hodgkin's lymphoma are nearing commercialization (18), which are shown in Table 1.

Table 1. Pharmaceutical Derived Proteins that are closest to commercialization for the treatment of human diseases (18).

Product	**Class**	**Indication**	**Company/ Organization**	**Crop**	**Status**
Various single chain Fv antibody fragment	Antibody	Non-Hodgkin's lymphoma	Large Scale Biology Corp	Viral vector tobacco	Phase I
CaroRx	Antibody	Dental caries	Planet Biotechnology Inc	Transgenic tobacco	Phase II
E.coli heat labile toxin	Vaccine	Diarrhoea	Prodigene Inc	Transgenic maise Transgenic potato	Phase I Phase I
Gastric lipase	Therapeutic enzyme	Cystic fibrosis, Pancreatitis,	Meristem Therapeutics	Transgenic maise	Phase II
Hepatitis B virus surface antigen	Vaccine	Hepatitis B	Arntzen Group Thomas Jefferson University/Polish Academy of Science	Transgenic potato Transgenic lettuce	Phase I Phase I
Human intrinsic factor	Dietary	Vitamin B 12 deficiency	Cobento Biotech AS	Transgenic *Arabidopsis*	Phase II
Lactoferrin	Dietary	Gastrointestinal infection	Meristem Therapeutics	Transgenic maise	Phase I
Norwalk virus capsid protein	Vaccine	Norwalk virus infection	Arntzen Group	Transgenic potato	Phase I
Rabies glycoprotein	Vaccine	Rabies		Viral vectors in spinach	Phase I

The advantages of using transgenic plants as bioreactors

There are many advantages of plant-based systems compared to other expression systems. Plant bioreactors are inexpensive. This is mainly due to plants producing biological materials using carbon dioxide, solar energy, and inorganic materials. Plant bioreactors are simple to scale up for agricultural use. Additionally, storage and transportation costs are reduced when recombinant proteins are produced in dry textures such as grains. The purification step is skipped when plant tissues containing recombinant protein are edible (6). As a result, certain costly biopharmaceuticals, such as human lysosomal enzymes, can be produced in plant bioreactors, which is especially useful in developing countries. Plant bioreactors cannot be contaminated by animal pathogens due to post-translational modifications (19).

The most significant advantage of transient expression systems is their production speed, which allows them to produce recombinant proteins in a matter of days. This platform is particularly well-suited for developing emergency vaccines and diagnostics, such as those used to combat novel influenza virus strains and SARS-CoV-2, which are required within a few weeks or months of confirming the virus gene sequence. Protein production capacities become scarce very quickly in such emergency scenarios because the production of other drugs and diagnostics cannot be stopped or delayed in the face of a new disease. Transient expression in plants provides a strategy for quickly closing production gaps (4, 20). Plants also have the advantage of administering various types of proteins, such as recombinant subunit vaccines, in the form of raw or partially processed fruits and vegetables (2).

Major barriers to broader market penetration and strategies to overcome them

There are fewer molecular farming products in the market compared to a large number of research studies due to some limitations of molecular farming, such as low plant productivity, high downstream processing costs, and slow translatability (4).

Low producibility: Many plant-based systems have low protein yields compared to industrial microbial and mammalian production platforms. Despite extensive research to optimize protein expression and stability, recombinant protein levels rarely exceed 100 g/kg fresh weight of plant tissue or per liter in suspension cell cultures (21). In general, protein yields from cell suspensions have been low, averaging 1–5 mg/L cell suspension culture. Furthermore, cell suspension cultures are frequently genetically unstable, whereas hairy root culture necessitates the use of expensive bioreactors and does not exploit the autotrophic capacities of the entire plant. An alternative to these methods is to use the plant's natural rhizosecretion mechanism, which in nature plays a role in nodulation, mycorrhizal colonization, growth inhibition of neighboring plants, nutrient acquisition from the soil, and defence against toxic metals (3). The yields of recombinant proteins produced by rhizosecretion are insufficient for commercialization. Several research groups have used various strategies to increase the yield of rhizosecretion, with varying degrees of success. These include the use of a root promoter or a plant signal sequence, the use of A. *rhizogenes* to induce hairy roots on transgenic tobacco plants, and the co-expression of the Bowman-Birk Ser protease inhibitor.

High costs of downstream processing: Downstream processing refers to the recovery and purification of the recombinant protein from plants (2). Because most host proteins are retained within the cell, the secretion of those proteins to the medium facilitates the production of recombinant proteins by microbes and mammalian cells. In theory, this applies to plant cell suspension cultures, hairy roots, and rhizosecretion systems based on whole plant hydroponic cultivation. Plant cells, on the other hand, secrete a number of host proteins into the medium, including proteases that can degrade the target recombinant protein, making the purification process more difficult (22, 23). To reduce the possibility of protease release, one approach is to develop secretion-based systems for recombinant proteins, which will make harvesting easier. For example, single chain Fv and monoclonal antibody heavy chain were

recovered from the surrounding growth medium of genetically modified tobacco cell suspensions, and *Agrobacterium rhizogenes*-derived hairy roots of tobacco were used to secrete assimilate (3).

Using seed-based expression is more beneficial than using a leaf-based expression which requires special attention. Seeds can be stored for longer periods as there are fewer chances of degradation of recombinant proteins expressed in the seed (6). The use of cell secretion systems may also be advantageous because there is no need to disrupt plant cells during replication, thereby avoiding the release of phenolic compounds. The use of affinity tags is another method of facilitating protein recovery. Protein tags should be removed after purification to restore the purified protein's structure to its native state (2). Another system is oleosin fusion technology, in which the recombinant protein gene sequence is fused to the sequence of an oil body-specific endogenous protein oleosin in rapeseed and safflower, and the protein is separated by an endoprotease digestion after purification (6).

Slow translation to applications: The third barrier is uncertain intellectual property and regulatory landscape compared to industrial microbial and mammalian cell expression systems, which have a long track record, particularly in biopharmaceutical manufacturing. As a result, the industry continues to view molecular farming as a risk and, in most cases, prefers to rely on tried and tested platforms. Molecular farming companies typically have Intellectual Property portfolios for their own expression systems, which should give industrial partners confidence. However, limiting industry partners to individual proprietary technologies effectively locks them in by the limitations of individual platforms, limiting their freedom to operate (4).

The Pharma-Planta Project is a group of scientists who study the intellectual property landscape in relation to plant-based pharmaceuticals (PMP). The goal is to make PMP end products and processes available and affordable to low- and middle-income countries. Because there is a significant patenting activity in the PMP arena, such analyses are critical to removing any potential barriers to ensuring freedom to operate (3).

Biosafety and the challenges of protein production and biomedicines in molecular farming

One of the most challenging issues is public concern about introducing genetically modified crops (2). Even though some of these products may pose a significant risk to the public, many would not pose a risk if they were introduced inadvertently. Many of the products in the pipeline are already in the food supply or are endogenous to humans (7). Lack of communication among the authorities dealing with research, biosafety and trade is an important issue that has hindered developments in molecular farming (2).

There are some environmental concerns about introducing transgenes into the food chain, which necessitates careful management and supervision (6). The long-term impact of molecular farming products on the environment is challenging to assess. An important concern is the food chain contamination with plant-made pharmaceuticals. This could happen as a result of the transfer of genetic material from transgenic plants to food crops. To avoid this, strict rules need to be put in place, such as geographically isolating the transgenic crop and growing in greenhouses instead of open fields and harvesting and processing transgenic plants with separate equipment or properly decontaminating the equipment if the same equipment is also used for food crops. Labeling genetically modified products are critical so that the consumer can choose based on their preferences (2, 24). Another source of concern is the use of agrobacterium in grain transformations, as grains are important crops in pharmaceutical protein production. These products can also lead to immune system reactions, which cause severe allergic reactions (6).

Future prospective of molecular farming

Current mammalian cell-based recombinant pharmaceutical manufacturing systems are incapable of meeting the demands. These systems' manufacturing capacity, safety, and reliability have been deficient on multiple occasions (14). The key to success in the future will undoubtedly be the level of expression of the recombinant protein in plants. The expression level affects the cost of growing, processing, extraction, purification, and waste disposal. Keeping the protein out of pollen can reduce inadvertent exposure to the environment, but this does not remove the possibility that the pollen will outcross with other plants and intermix with food crops. The regulatory agencies impose physical isolation requirements to prevent this from occurring (7).

Biotechnology companies have aimed to commercialize the antibodies produced in plants. It has been estimated that the increasing annual need for secretory IgA will be 13%, and a rate of $25 billion was predicted as the annual income for producing IgA in crops. The challenges include the difficulty of low yield of protein, the possibility of harmful effects on the function/performance of proteins due to the differences in glycosylation patterns, and the severe potential impact of expressing plants of biomedicine plants on the environment (6).

The use of plants in pharmaceutical manufacturing, in general, appears to be improving, especially with the recent approval and licensure of Protalix's carrot cell that produced Gaucher disease therapeutic enzyme. The prospects for animal vaccines in general, as well as possibly human therapeutic vaccines, appear promising. The length and rigor of the human prophylactic vaccine developmental and clinical testing path compared to animal and human vaccines are a massive obstacle to the commercial production of novel bio-farmed vaccines (14, 25).

Transient expression in plants provides a strategy to close production gaps quickly in an emergency. Transient expression allows plants to be grown while the pathogen's genome sequence is being investigated. The plants can then be ready for protein production as soon as antigen sequences are available. Therefore, many academic and industrial groups use this technique to produce diagnostics and therapeutics against SARS-CoV-2 (20).

Conclusion

The goal of molecular farming is to produce large quantities of active and safe pharmaceutical proteins at a low cost. Nowadays, gene transfer methods in plants have advanced significantly as a result of scientific advances in the field of biotechnology. Compared to other microbial and animal expression systems, these transgenic plants have several advantages in terms of ease of production, cost, safety, and producing pharmaceutical biomolecules. So far, many valuable pharmaceutical proteins and antibodies have been produced using this method, which has significantly aided patient treatment, particularly in developing countries where the production and preservation costs of such medicines are prohibitively expensive. However, there are some disagreements, such as public acceptance, transgene escape and biosecurity, clinical and commercialization investigations of products, and so on, which have made it a complex area, but it is hoped that with the efforts of researchers and scholars, molecular farming will achieve great success in the near future.

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Letter to Editor

Academic Recognition of Systematic Reviews and the Evidence-based Research Approach in Postgraduate Health and Clinical Sciences in Sri Lanka - Can the University of Peradeniya Take the Lead?

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One of the main ways to minimize waste in research and maximize the likelihood of research having an impact is to justify planned research based on an assessment of the global body of evidence already available. This requires identifying or conducting a systematic review within which the planned research question clearly addresses existing evidence gaps while summarizing and synthesizing existing knowledge. Such an approach ensures that research is evidence based. Evidence-Based Research (EBR) is the systematic and transparent use of prior research to inform a new study to answer questions that matter valid, efficient, and accessible (1-3). The EBR approach promotes i) well-defined research questions addressing the uncertainties within the current evidence base and ii) research questions answering the needs of end-users. If this is part of any planning of new research, only studies meeting research gaps and societal needs will be prepared and published, and thus redundancy will be avoided. In addition, the EBR international network promotes a more efficient production, updating and dissemination of systematic reviews (1-3).

However, as in many other countries (4), the EBR approach is still far from the norm in Sri Lanka. Barriers to using an EBR approach include lack of academic recognition of systematic reviews (5), underdeveloped systematic review methodological expertise, lack of access to citation databases and full-text papers only accessible through paywalls. Given the importance of the EBR approach, universities can play an essential role in removing barriers to its implementation. For example, universities could improve the academic recognition of systematic reviews in under and postgraduate programs (6).

With its academic and research diversity and more than 12000 undergraduate and 4000 postgraduate students, the University of Peradeniya can make revolutionary changes in promoting EBR in Sri Lanka.

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Promoting and supporting the use of systematic reviews would help staff and students' capacity to plan, design, conduct, analyze, and report systematic reviews.

Specific activities which will be helpful include, i) organizing lectures/ lunch talks for academic staff on the importance of systematic reviews, ii) training on systematic review methodology including train the trainer programs, iii) organizing annual workshops on systematic review methodology for academic staff members, research supervisors, and higher degree students, iv) obtaining access to the electronic databases and required software, v) organizing annual seminars for academic staff and supervisors on importance of systematic reviews in research projects, including higher degree programs, vi) training librarians on database search and on building systematic search strategies, vii) training of statisticians to support meta-analyses and meta-regression, viii) promoting systematic reviews as dissertation projects (or chapters within PhDs), ix) undergraduate and postgraduate level training through workshops/ seminars, x) encouraging conduct of systematic reviews in higher degrees, xi) establishing an EBR national center/ National Centre for Synthesis of Evidence and/or Cochrane affiliation at the University of Peradeniya, xii) promoting participation in international training and fellowships, and making international collaborations through the established EBR national center/ National Centre for Synthesis of Evidence and/or Cochrane affiliation. To make EBR a reality, the coordination between the faculty education units, higher degree committees, faculty libraries, higher degree advisory and faculty boards, heads, deans, the vice-chancellor, and the systematic review and national centers of evidence synthesis around the globe is required. While some practicalities such as database access and software access remain challenging, this proposal can be supported by internal and external resource personnel (international EBR Network, Cochrane teams (e.g., Cochrane Ireland), Evidence synthesis Ireland). Coordination through an International Advisory Group would assist in identifying the potential barriers, challenges and funding.

The benefits of the academic recognition of systematic reviews and evidence synthesis and the EBR approach include potential solutions to many obstacles in Sri Lanka in achieving excellence in scientific research and publications (5). More relevant and needed research will be performed using systematic reviews when justifying and designing new studies. The scarcity of high-quality systematic reviews submitted and published by the University of Peradeniya and Sri Lankan academics can be better managed. High-quality systematic reviews are essential in presenting the synthesis of evidence for better decision-making. Further, it will help the academic and research staff to develop their scientific writing skills, improve their methodological expertise and increase their networking capacity and collaborations. In the long-term, we as a university can have a national center for evidence synthesis, which could become a critical source of national and international research collaborations.

We leave below a statement published by The Lancet (2005) (7) and invite and encourage all the academic and research staff to take your lead in actively promoting and utilizing the systematic review and the EBR approach as a part of your research project/s.

“Unnecessary and badly presented clinical research injures volunteers and patients as surely as any other form of bad medicine, as well as wasting resources and abusing the trust placed in investigators by their trial participants. Those who say that systematic reviews and meta-analyses are not “proper research” are wrong; it is clinical trials done in the absence of such reviews and meta-analyses that are improper, scientifically and ethically. Investigators and organizations who undertake and coordinate reviews and meta-analyses now need the funding and recognition they deserve if public trust in biomedical research is to be maintained and resources used in an effective way” (7).

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Letter to Editor

Food Security in Sri Lanka During COVID19 Pandemic

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The COVID19 pandemic has presented an unprecedented challenge to many low and middle-income countries' public health, social security, and food security (1). Food insecurity is a situation that exists when people do not have secure access to sufficient amounts of safe and nutritious food for normal growth, development and active healthy life (2).

In Sri Lanka, the first case of COVID19 was reported in late January 2020 (3). With COVID19 cases continuing to rise, the World Food Program raised food security concerns among vulnerable sections in Sri Lanka due to the impact and control response of the COVID19 outbreak (4). In 2019, Sri Lanka was ranked 66 among 113 countries in the global food security index, which indicates that food security was an area that required intervention even prior to the COVID19 pandemic (5).

During the prolonged curfew imposed in mid-March 2020, Sri Lanka began to face the impacts of COVID19 on food systems (6), in a dual shock on agriculture markets which has affected both supply and demand (7). Increased levels of food loss and wastage occurred due to market closure and blockages to transport routes (5). According to the Central Bank of Sri Lanka's annual report, weaker earnings from tea, seafood, and other agricultural exports in 2020 exhibited a decline in earnings by 5.1% compared to 2019 (8). The sudden imposition of curfew and its continuation has confined the movement of people to restricted hours. As a result, huge queues occurred after lifting the curfew (5). Year-on-year inflation increased to 6.0% in August 2021 due to high food inflation (11.5%) (9). Normal crop cultivation and harvesting were adversely impacted due to being advised to stay home (42%), being unable to purchase inputs (33%), and poor demand in the market (56%) (10). According to a study, half of the households were either moderately (36%) or severely (14%) affected by food insecurity between December 2020 and February 2021 (10).

To counteract the adversities faced by the COVID19 pandemic, Sri Lankan Government implemented numerous measures to address immediate supply and demand side issues (6). The government allowed farmers to continue with their operations, and approval of the transport of vegetables during curfew hours was helpful to minimize losses (11). Packets of seeds were distributed free of charge in some areas (11). Notably, the "Saubhagya" National Program on Harvesting and Cultivation was launched to develop one million home gardens islandwide (11). However, the World Bank's rapid phone surveys done in 2021 showed that around 44% of households had worried about running out of food. Among this group, more than 80% reduced consumption or ran out of food (12). Also, food price inflation in Sri Lanka remained at elevated levels, making food less affordable (6). A study published in July 2021 showed that, based on the cost assessment of basic breakfast or lunch, the highest rises have been in Sri

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Lanka and Pakistan, with over 40% during COVID19. The average cost of a lunch for a family of four has increased by 44.5% based on a table including chicken, eggs, rice and vegetables (13). Also, despite the broad outreach of the policy response to support livelihoods, more than 45% of households indicated that further distribution of food and cash transfers would be helpful (12).

Adapting alternative inputs and marketing strategies, promoting innovative technology, providing immediate financial support, and implementing intervention strategies tailored to farmer heterogeneity would improve the sector's prosperity in a crisis situation (14). Even if the spread of COVID19 begins to ease shortly, we cannot pass it off as a temporary shock. Hence, it is essential to strengthen the food system to face future blows. Furthermore, attention should be paid to developing strategies to efficiently manage national food reserves, which is of utmost importance in the backdrop of a pandemic.

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