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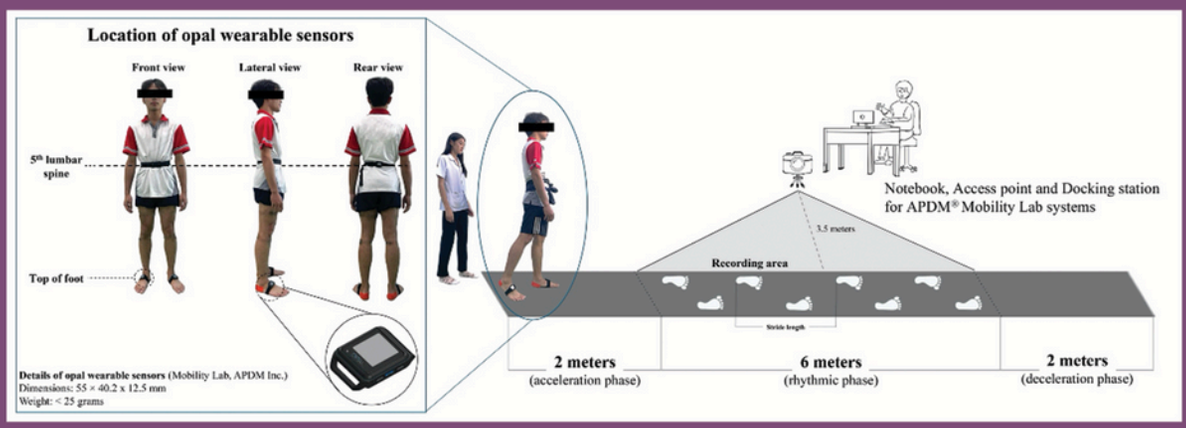

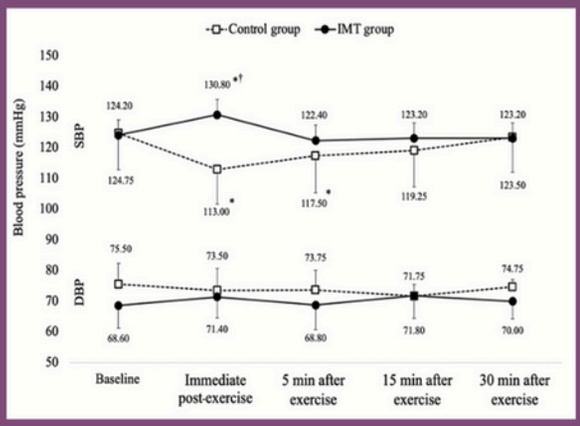

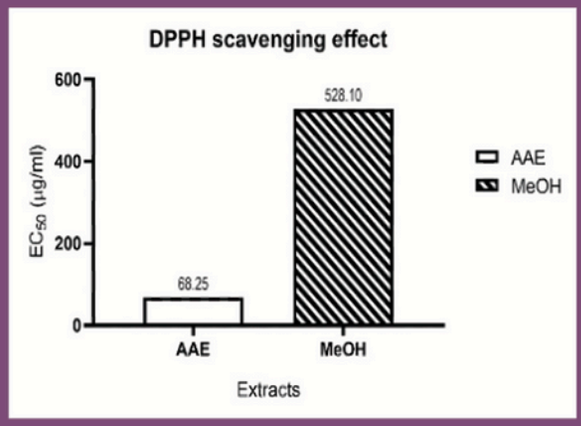

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Post-exercise blood pressure responses and cardiac stress after inspiratory muscle training in COPD patients: a pilot study

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KEYWORDS

Respiratory muscle training;
Rate pressure product;
Heart rate variability;
Cardiovascular response;
Blood pressure.

ABSTRACT

Acute responses following a single bout of exercise have been shown to predict blood pressure regulation and cardiovascular adaptations. Inspiratory muscle exercise (IMT) has been utilised in pulmonary rehabilitation programmes for COPD patients, but little is known about the immediate effects of IMT exercise on post-exercise blood pressure and cardiac responses. The current study aimed to investigate acute post-exercise blood pressure, cardiac autonomic, and myocardial oxygen demand responses to a single session of IMT among COPD individuals. Ten male COPD patients, with an average age of 65.44 ± 4.38 years, volunteered for the study. Subjects underwent the IMT protocol at 60% maximal inspiratory pressure as an inspiratory load (6 breaths/set, 1-minute rest between sets, 5 sets), while the control group subjects performed breathing without inspiratory load. Blood pressure and heart rate variability were measured before and immediately, 5, 15 and 30 min after the exercise. Systolic blood pressure in the IMT group was significantly higher than in the control group immediately after exercise. Sympathetic cardiac autonomic modulation and sympathovagal balance also showed similar responses to systolic blood pressure. The rate pressure product index of myocardial oxygen demand and heart rate significantly increased from baseline compared to immediate post-IMT exercise. However, all variables returned to the baseline values within 15 min following exercise, and no adverse effects were reported after the IMT programme. Our data suggest that single bouts of IMT sessions neither elicit post-exercise hypotension nor a high cardiac autonomic response in patients with COPD. Thus, moderate-intensity IMT exercise with a short duration appears to be safe for this population.

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Introduction

Chronic obstructive pulmonary disease (COPD) is a common chronic respiratory disease that is the leading cause of mortality worldwide[1]. COPD patients commonly present with respiratory muscle dysfunction, leading to physical activity intolerance[2].

Inspiratory muscle training (IMT), a series of resistive breathing exercises that aim to promote respiratory muscle strength, is considered part of a pulmonary rehabilitation programme. Previous evidence has confirmed the efficacy of IMT on strengthened respiratory muscle, attenuated dyspnoea and improved exercise performance in patients with COPD[3-5]. Apart from the beneficial effect on the pulmonary system, the blood pressure lowering effect of IMT has also been investigated according to autonomic cardiovascular modulations following the implementation of IMT exercise[6]. A recent meta-analysis demonstrated that long-term IMT intervention decreased systolic blood pressure and diastolic blood pressure in healthy individuals and hypertensive patients and was in part mediated by an alteration in cardiac autonomic activity[6]. In addition, it is suggested that the chronic lowering of resting blood pressure following exercise training may be due to a repeated post-exercise hypotension (PEH) response[7,8]. Focus on acute exercise responses, a single bout of IMT exercise with a load of 30% of maximum inspiratory pressure (MIP) for 15 min, induced post-exercise hypotension together with improved heart rate variability (HRV) in young smokers[9]. By contrast, no significant change in blood pressure and cardio-autonomic activity has been reported after acute IMT at 60% maximum inspiratory pressure (PI_{max}) as a resisted load in patients with type 2 diabetes[10]. A recent study showed that the acute effect of IMT at an intensity of 70% MIP had no influence on systolic blood pressure, diastolic blood pressure, HRV or inflammatory markers in obstructive sleep apnoea subjects[11]. As mentioned, the acute effect of IMT exercise on blood pressure response is controversial.

The rate pressure product (RPP) is a marker of myocardial oxygen demand. Moreover, increased RPP also predicts cardiovascular mortality[12-14]. It is well known that cardiac autonomic impairment has been observed in COPD patients and is associated with the development of cardiovascular disease[15,16]. However, little is known about the post-exercise blood pressure, cardiac autonomic responses, and myocardial oxygen demand during a single session of inspiratory muscle exercise in COPD patients. Thus, the present study focused on investigating the acute effect of IMT on post-exercise blood pressure responses, HRV, and rate pressure products in COPD patients. We hypothesised that IMT would alleviate blood pressure and myocardial oxygen demand; these effects may be related to cardiac autonomic modulation.

Materials and methods

Participants

This study recruited 10 pilot COPD patients aged 40–70 years with GOLD stages II–III, following the Global Initiative for Chronic Obstructive Lung Disease guidelines[17]. All patients were clinically stable with absence of respiratory exacerbation within eight weeks prior to the study and had not been involved in any exercise programme. Participants were excluded if they had neurological or musculoskeletal problems that could limit the exercise protocol. We declare that all participants read and signed an informed consent form for study participation, and this study was approved by Khon Kaen University institutional review board (HE622217).

Study design

The participants were randomly divided into either the control or the IMT group using the stratified randomisation method. One week before the measurement, the participants were familiarised with the experimental procedures, and IMT breathing was supervised by a physical therapist[18]. Furthermore, participants were advised to refrain from alcohol, caffeine beverages

and vigorous exercise in the 24 hours preceding testing.

On the test day, the participants were asked to rest for 10 min before the experiment, as described in figure 1. First, baseline measurements were collected: the outcome parameters, including BP, HR, HRV, RPP, and oxygen saturation (SpO_2). Participants in the IMT group received a single session of IMT exercises, while the control group received breathing exercises. After the exercise session, all the participants'variables were measured immediately after the exercise and in the recovery period at 5 min, 15 min, and 30 min after the exercise. Additionally, adverse effect symptoms were recorded for the purpose of investigating the safety of the IMT protocol.

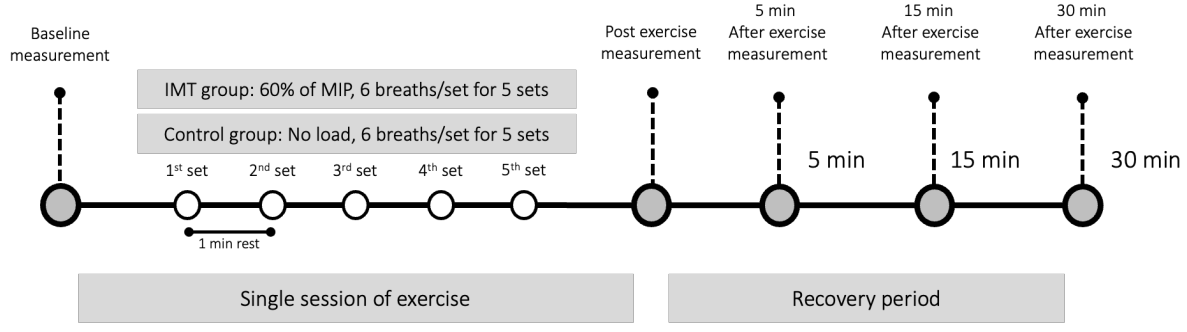

Figure 1 Study protocol and outcome measurement timeline.

Inspiratory muscle training protocol

Prior to the IMT exercise, the MIP was measured for each individual using a MicroRPM respiratory pressure meter (PhysioParts, UK). Participants performed three trials of maximum inspiratory manoeuvre with a 1-minute rest interval between tests. The best of the three trials was used as resistant load, with values that varied no more than 5% of the highest value[19].

The IMT group performed IMT exercises using threshold inspiratory muscle trainer equipment (Koninklijke Philips N.V., Netherlands), and the IMT protocol was set as previously described[9,20]. After attaching the mouthpiece and nose clip, participants were instructed to inhale from the residual volume to overcome the resisted spring load at 60% of their MIP. Participants completed IMT for six breaths per set, for five sets with 1-minute rest between each set. On the other hand, the control group received a single session of breathing exercises with the same protocol, except for no inspiratory load. During the training session, all participants were recommended to maintain diaphragmatic breathing and inhale with maximum effort against load with the same resting breathing frequency. In addition, participants were instructed to stop the exercise if adverse effects occurred, including increasing breathlessness, chest discomfort, dizziness, or near syncope[21].

Outcomes

BP, HR, and SpO_2 were measured using a vital sign monitor (Nihon Kohden Vismo, Japan). The measurement procedure followed the standard guidelines of the American Heart Association[22]. BP was collected three times with at least a 1-minute rest interval. The average value was recorded and used for further analysis.

HRV was measured in a quiet room according to previous guidelines[23]. It was continuously assessed by the R-R interval via electrocardiogram (EKG) using a computer and Acknowledge data collection software (Biopac Systems, USA). The EKG was recorded at 5 min before IMT exercise,

5 min immediately after IMT exercise and at 5–10, 15–20 and 30–35 min after IMT exercise. The time domain (standard deviation of all normal-to-normal R-R intervals [SDNN]) and the frequency domain (high frequency normalised unit [HF], low frequency normalised unit [LF] and low frequency to high frequency ratio [LF/HF]) of the HRV were averaged and analysed for each participant[24]. SDNN reflects all the cyclic components responsible for variability in the period of recording; therefore, it represents total variability. The HF was used as marker of vagal modulation, the LF as index of sympathetic modulation, and the LF/HF represented sympathovagal balance[23].

RPP, a marker of myocardial oxygen demand and cardiac work[25,26], was calculated by multiplying systolic blood pressure (SBP) and HR. During and after completing the exercise, the participants were asked about incidents of adverse effect symptoms[27].

Statistical analysis

Statistical analysis was performed using Statistical Package for Social Sciences (SPSS) version 26 and the Shapiro-Wilk test for normality of distribution was applied. Continuous data were presented as mean ± standard deviation. The data were analysed using a two-way repeated measure ANOVA, followed by Tukey's post hoc test to evaluate specific differences between means. An alpha level of < 0.05 was considered statistically significant.

Results

Participant characteristics

All participants were male, with an average age of 65.44 ± 4.38 years. Baseline characteristic data for each group are summarised in table 1. No statistical differences were observed between the groups in terms of their characteristics.

Table 1 Participant characteristics

	Control group (n=5)	IMT group (n=5)
Age (year)	62.50 ± 2.38	67.80 ± 4.38
Weight (kg)	56.25 ± 4.27	48.98 ± 6.33
Height (cm)	161.1 ± 8.60	160.40 ± 5.03
BMI (kg/m^2)	21.77 ± 2.69	18.98 ± 1.62
MIP (cmH_2O)	59.50 ± 20.04	58.20 ± 10.80

Note: Data present as mean ± SD.

Abbreviation: BMI, Body mass index; MIP, Maximum inspiratory pressure; kg, Kilogram; cm, Centimeter; kg/m^2, Kilogram per square meter; cmH_2O, Centimeter of water.

Blood pressure responses to inspiratory muscle training

Figure 2 shows the SBP and DBP responses before and after IMT. No significant difference in baseline blood pressure was observed between the control and IMT groups. At immediate post-exercise, SBP was significantly elevated following IMT compared with the initial value, while the control group showed a significant reduction in SBP. In addition, SBP was higher after a single bout of IMT than the control condition during immediate post-exercise (p-value < 0.05), and then returned to resting baseline values within 15 min in both groups. In contrast, DBP was not significantly altered at any time point either with the IMT exercise or the control group (Figure 2).

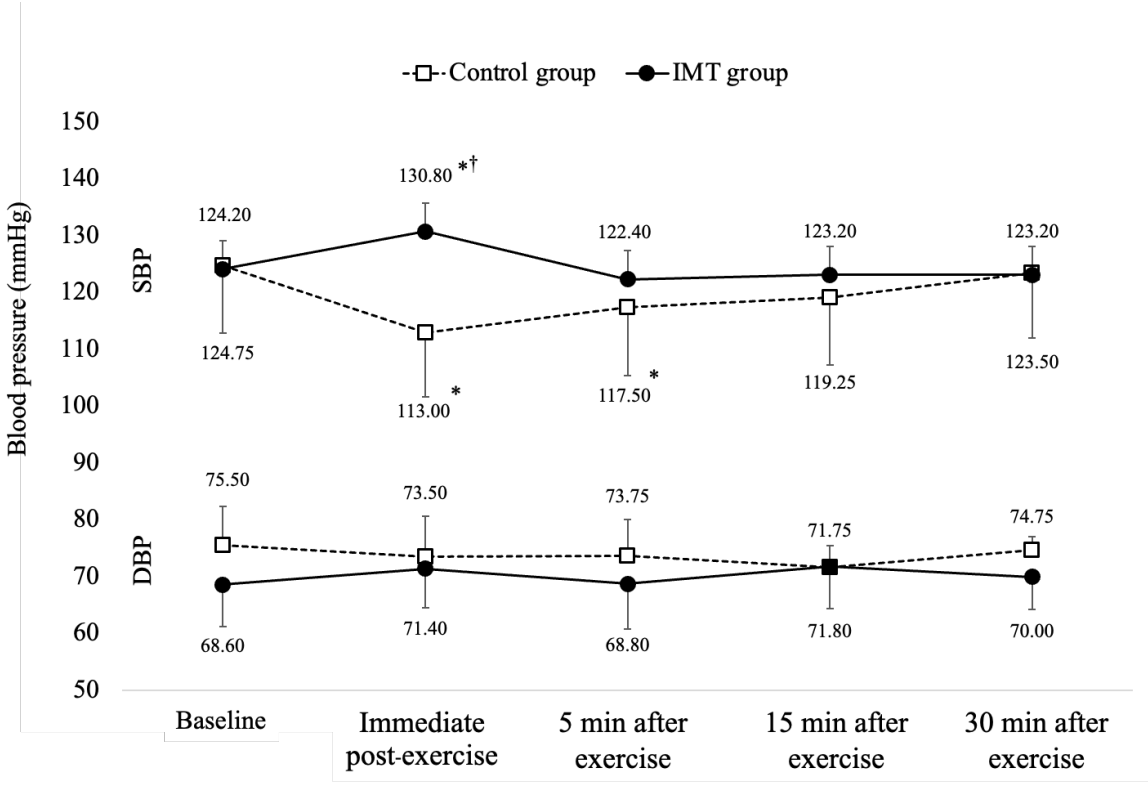

Figure 2 Time course of change in SBP and DBP in the control group and IMT group at baseline, immediate post-exercise, 5 minutes, 15 minutes, and 30 minutes after exercise.

Note: * *p*-value < 0.05 vs. Baseline; † *p*-value < 0.05 vs. the control group.

Abbreviation: SBP, Systolic blood pressure; DBP, Diastolic blood pressure; mmHg, Millimeter of mercury.

Heart rate and rate pressure product responses to inspiratory muscle training

In response to the IMT exercise, HR was increased during the immediate post-exercise compared to baseline values (*p*-value < 0.05, Table 2). However, HR remained unchanged in the control group. The post-hoc test indicated that HR was not significantly different between the conditions. A significant increase in RPP was observed within the IMT group during immediate post-exercise; this was unchanged within the control group. In addition, the inter-group analysis revealed no significant differences between the IMT and the control groups (*p*-value > 0.05). Indeed, HR and RPP returned to resting values during the recovery period after the IMT session (Table 2).

Autonomic responses to inspiratory muscle training

Table 2 presents the HRV responses to the IMT exercise among the COPD patients. The IMT group exhibited a significant within-group reduction in SDNN and HF during immediate post-exercise compared to the rest (*p*-value < 0.05, Table 2). LF and LF/HF were significantly increased responses to a bout of IMT exercise. On the other hand, the control group showed significantly lower LF when compared to the baseline values (*p*-value < 0.05).

The analysis between the groups indicated that IMT exercise resulted in lower HF and higher LF and HF/LF values immediately post-exercise compared to the control group. Accordingly, no significant differences between groups in HRV were observed at 5, 15 and 30 min after exercise.

SpO_2 and adverse effects

There was no significant change in SpO_2 throughout the time period in both groups (*p*-value > 0.05, Table 2). Furthermore, no adverse events were reported in any of the patients during the exercise sessions.

Table 2 Variables in control and inspiratory muscle training (IMT) group at baseline, immediate post-exercise, and recovery period

Variables	Group	Baseline	Immediate post-exercise	5 min after exercise	15 min after exercise	30 min after exercise
HR (bpm)	Control	82.25 ± 15.28	79.25 ± 12.58	81.25 ± 14.59	79.25 ± 16.01	79.25 ± 15.09
	IMT	71.60 ± 6.35	78.00 ± 9.08*	72.60 ± 10.50	69.20 ± 9.36	69.20 ± 7.69
RPP	Control	9865.20 ± 2109.36	9403.60 ± 2061.71	9802.40 ± 2282.50	9648.80 ± 2407.81	9895.20 ± 2338.14
(mmHg.bpm)	IMT	8919.20 ± 1261.10	10238.60 ± 1751.12*	8925.00 ± 1724.51	8557.20 ± 1537.21	8570.40 ± 1611.45
SDNN (ms)	Control	30.15 ± 5.50	31.83 ± 14.18	26.15 ± 8.51	27.98 ± 7.47	26.99 ± 9.81
	IMT	33.42 ± 17.69	23.04 ± 12.15*	28.10 ± 11.16	35.58 ± 19.87	35.47 ± 15.58
HF (n.u.)	Control	64.07 ± 5.04	72.86 ± 3.35	66.32 ± 4.92	62.50 ± 9.21	54.56 ± 14.02
	IMT	58.07 ± 10.11	34.70 ± 21.36*†	52.65 ± 16.20	61.23 ± 10.41	58.89 ± 11.82
LF (n.u.)	Control	35.47 ± 4.88	26.72 ± 4.21*	38.50 ± 14.65	30.87 ± 6.33	30.46 ± 5.86
	IMT	40.96 ± 8.99	66.78 ± 21.92*†	45.52 ± 15.45	37.49 ± 8.35	35.79 ± 10.37
LF/HF	Control	0.56 ± 0.13	0.40 ± 0.14	0.60 ± 0.29	0.51 ± 0.16	0.58 ± 0.14
	IMT	0.75 ± 0.35	2.75 ± 1.81*†	1.05 ± 0.76	0.64 ± 0.26	0.64 ± 0.29
SpO_2 (%)	Control	96.75 ± 0.96	98.00 ± 0.00	96.25 ± 1.50	96.50 ± 1.29	97.00 ± 1.15
	IMT	96.60 ± 2.60	97.00 ± 3.39	96.80 ± 3.42	96.60 ± 3.05	96.80 ± 2.86

Note: Data present as mean ± SD; * significant difference within group when compared with baseline (*p*-value < 0.05); † significant difference between group (*p*-value < 0.05).

Abbreviation: HR, Heart rate; RPP, Rate pressure product; SDNN, Standard deviation of all normal to normal R-R intervals; HF, High frequency normalized unit; LF, Low frequency normalized unit; LF/HF, Low frequency to high frequency ratio; SpO_2, Oxygen saturation.

Discussion

The present study examined changes in post-exercise blood pressure, cardiac autonomic and myocardial oxygen demand in response to one session of inspiratory muscle training in moderate-severe COPD individuals. We demonstrated that no post-exercise hypotension occurred after moderate-intensity IMT exercise among COPD patients. In addition, acute IMT exercise in COPD patients did not provoke cardiac overload as assessed by RPP in comparison with control conditions. Lastly, there were no adverse events, such as increased breathlessness, chest discomfort, dizziness, or near syncope, during the IMT session.

Our results are in agreement with those in a previous study that reported no evidence of post-exercise after completing a single bout of IMT exercise. Findings by Tanriverdi et al[28] exhibited a rise in mean blood pressure (~5%) after performing an acute IMT at an intensity of 60% MIP in healthy young participants. Furthermore, an acute session of inspiratory exercise using a load of 70% MIP in obstructive sleep apnoea subjects did not induce significant changes in blood pressure and heart rate[11]. In contrast, previous studies have demonstrated that breathing exercise at 30% MIP attenuated systolic blood pressure (~13%) and improved cardiac autonomic modulations in young smokers[9]. Thus, in the current study, the

elevation of SBP (~5%) was observed immediately after IMT exercise. A possible explanation relates to the respiratory metaboreflex activation during exercise, which is stimulated by the accumulation of metabolite products from respiratory muscle fatigue[29]. Metaboreflex activation has been consistently reported to increase blood pressure by increasing sympathetic outflow. Previous research has shown that diaphragm muscle fatigue is associated with sympathetic overactivity, leading to increased HR and BP[30]. Indeed, our present study showed that the rise in systolic blood pressure lasted for 5 min following an IMT session using 60% MIP, suggesting that the acute IMT exercise did not induce major haemodynamic changes in moderate-severe COPD participants.

Cardiac autonomic imbalance is considered a potential predictor of cardiovascular events and has also been observed in individuals with COPD. A previous study reported a reduction in HVR among COPD patients, including low vagal modulation, with a predominance of sympathetic activity[31,32]. IMT has been described as an exercise therapy that may improve heart autonomic control according to the interactions of breathing patterns and HR regulation[33]. In older adults, short-term exercise breathing (15 breaths at a load of 30% MIP) induced beneficial changes in the HRV component, as indicated by enhanced LF and attenuated HF[34]. In contrast, the findings of this study revealed that in COPD individuals, a single bout of IMT session with 60% MIP increases sympathetic and decreases parasympathetic activity at 5-minute post-exercise and returns to baseline values at 15-minute post-exercise. Similar to our results, Schein et al. also showed a decrease in vagal modulation following an inspiratory exercise with 60% of PI_{max} in subjects with type 2 diabetes[10]. These findings may be explained by the influence of loading intensity on cardiac autonomic modulation. Prior studies reported that low-intensity IMT produced a greater parasympathetic modulation than high-intensity IMT[24,28]. Nevertheless, the present study showed that the HRV indices were not different in responses to IMT compared to the control group during 5–30 min post-exercise. A previous study also found that HF and LF components were unchanged during acute resistive load breathing using 70% MIP in moderate to severe OSA individuals, suggesting the clinical safety of implementing IMT in this population[11]. These contradictions in blood pressure and cardiac autonomic responses following IMT might be influenced by the resistance load, initial respiratory muscle strength, and clinical characteristics of participants[28,35].

Regarding myocardial oxygen consumption, this study did not observe significant differences in RPP between the evaluated conditions. We suggest that IMT does not provoke cardiac overload in subjects with moderate and severe COPD. RPP is often estimated during exercise as a proxy of myocardial requirements as well as tolerance for exercise[36]. In addition, RPP is a strong predictor of cardiovascular and all-cause mortality. These data are in accordance with those in the study of Ramos et al[37] who found no major haemodynamic responses in elderly patients submitted to two sets of IMT sessions at 30% of MIP resisted load. Thus, a single bout of moderate-intensity IMT exercise seems to be safe and well tolerated for moderate to severe COPD patients.

Conclusion

A single session of IMT at 60% of MIP in COPD individuals fails to induce PEH. However, for COPD patients, a short IMT session seem to be clinically safe.

Take home messages

Acute IMT appears to be clinically safe and well tolerated in subjects with COPD and it could be used as an adjuvant exercise to increase patient compliance.

Conflicts of interest

The authors declare no conflict of interest.

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Assessing the acceptability of an Automated Health Records Information System in the universities: a technology acceptance model approach

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KEYWORDS
Automated health records information system;
Technology acceptance model;
University clinics.

ABSTRACT

This research assessed the acceptability of the Automated Health Records Information System in a state university clinic located in Cebu, Philippines, and aimed to enhance healthcare management in the digital era. Employing the Technology Acceptance Model, the study examined user perceptions and satisfaction with the software, providing insights into its effectiveness in improving workflow efficiency and patient care. The study employed a weighted mean utilizing a five-point Likert scale to evaluate the acceptability of the software and a four-point scale for expert validation. A descriptive correlational method was utilized, applying Pearson r to analyze the degree of correlation. The study revealed that the software was highly acceptable based on perceived usefulness, ease of use, intention to use, and actual use, as rated by the respondents. Moreover, the technical requirements of the software, including design, features, and program content, were also positively rated. The null hypotheses were not rejected as there were no significant interrelationships among the four acceptability variables, indicating that other factors, such as the type of institutional support, user's characteristics, experiences, and skills, may influence the inter-correlation acceptability of variables. Respondents highlighted challenges in software navigation and expressed the need for comprehensive training. Despite these challenges, the study strongly recommended the acceptability and adoption of the Automated Health Records Information System to enhance healthcare delivery within university clinics, emphasizing its role in seamlessly managing health records for all university personnel and students, in alignment with broader institutional objectives aimed at improving overall well-being and organizational efficiency.

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Introduction

The COVID-19 pandemic had pushed healthcare institutions worldwide to their breaking point, revealing significant challenges in the ability of current healthcare systems to effectively manage the crisis. This global health emergency tested the capacities of both public and private healthcare services, highlighting weaknesses in providing adequate care. The absence of standardized organizational and treatment protocols exacerbated the strain on healthcare systems, hindering effective responses[1]. In a study conducted in Africa, where many nations are low-income countries, the coronavirus had a devastating impact due to already weak healthcare systems[2]. The study underscored the incapacity of African healthcare systems to address the pandemic, leading to unprecedented deaths during its peak.

As computer technology and software continue to advance, the automation of the healthcare system with the Internet of Things (IoT) was ready to assume a pivotal role in mitigating such risks[3]. The integration of IoT into health-care systems had been successfully implemented, yielding precise outcomes. Amid the COVID-19 crisis, the automation of health monitoring systems had become crucial in the medical field. Researchers and medical professionals found the programme to be very helpful in expediting COVID-19 treatment and preventive efforts. The Internet of Things-based automated health monitoring programme complied with modern technological norms and offered a better method of providing healthcare[4]. In order to manage data and additional information at the quick speed required under these circumstances, information technology was essential.

A Health Information System (HIS) comprised interconnected elements that gathered, processed, stored, and disseminated data and information. This functionality aided in decision-making, facilitated the management of health organizations, and improved healthcare applications[5]. Health information technology had the capacity to revolutionize healthcare delivery by providing information where it was required and reshaping healthcare to prioritize the consumer. This transformation could occur without significant regulatory changes or disruptions in the industry. It enabled improved quality, safety, and responsiveness to consumer needs, as well as enhanced efficiency, reduced waste and increased accessibility to care[6].

According to the National Association of School Nurses (NASN), electronic health records (EHRs) were crucial for registered professional school nurses. EHR platforms that adhered to modern nursing standards empowered school nurses to enhance healthcare coordination, quality, equity, safety, efficiency, and effectiveness. These capabilities contributed to maximizing the educational potential of school-age youth[7].

The medical personnel were responsible for keeping track of the employees' and students' medical records to provide a healthy atmosphere. All registered nurses in private and public school institutions had to access EHRs software platform that complied with confidentiality, security, and privacy regulations. School nurses were required to maintain accurate and secured student health records, following standardized nursing practices[8,9]. EHRs were identified as the most effective and secure method for managing this information[10].

The number of software programs available to users increased due to the fast development of technology. However, not all software programs were embraced and utilized by users. The Technology Acceptance Model (TAM)[11] emerged as a suitable choice, given its established focus on user's perceptions of technology's usefulness, ease of use, intention to use, and actual usage[11].

The TAM outlined how individuals accepted information systems. TAM suggested that user's adoption of a technology was determined by their perception of its utility in task completion and ease of use,[12-14] asserting that the adoption of information technology benefitted individuals and organizations short- and long-term by improving

performance, saving time and money, and offering convenience, while innovations like wearables, virtual reality, and IoT transformed healthcare, giving patients more mindful, patient-centric choices.

According to the TAM, people undergo a three-step process when deciding whether to embrace new technology. Initially, individuals evaluated the design of the technology to gauge its usability and potential advantages. These assessments then shape their attitudes towards using it. Ultimately, their emotions played a pivotal role in deciding whether to embrace or decline the technology[11,15]. A study conducted by Davis FD[15] and further supported by Ajzen I[16] found that the attitude towards behavior, termed behavior intention, played a crucial role in technology acceptance. This emotional assessment of potential consequences is more influential in determining actual behavior than mere behavioral intention.

The study aimed to evaluate and adopt an Automated Health Records Information System (AHRIS) for digitizing medical records, updating them in real-time, validating the software's technical requirements, and contributing to the advancement of healthcare technology within university clinic. Its significance lay in its focus on addressing the urgent need to enhance healthcare management in educational settings, particularly in the digital age when efficient record-keeping was crucial for both patient care and operational efficiency.

Materials and methods

Participants and procedures

The study utilized a quantitative approach with a descriptive-correlational design involving two respondent groups. The first group comprised 101 employees, including two clinic nurses authorized to access the system, organic teaching personnel, organic non-teaching personnel, part-time teachers, and contract-based employees. The second group comprised 300 students from a range of programs, such as Industrial Technology, Hospitality Management, Elementary Education, Secondary Education, Marine Engineering, and Fisheries and Allied Sciences.

The study was conducted at one of the state universities in Cebu, Philippines, which was dedicated to the holistic development of all stakeholders through various educational programs and the integration of fundamental values for global competitiveness. Three computer experts, each with extensive experience in software development and system security, authenticated the technical aspects of the software. They ensured that the system met industry standards, including compliance with the Data Privacy Act of 2012 (Republic Act No. 10173), ISO/IEC 27001 for information security, TESDA's e-Governance Standards, and the National ICT Standards set by the DICT, verifying that the system was robust enough to handle the anticipated data load while maintaining data privacy and security.

Every participant in the study completed a standardized questionnaire with three components. The first section gathered data on demographics. Using the TAM, the automated health records information system's acceptability was evaluated in the second phase. The third section addressed challenges encountered by participants while navigating the system. The clinic personnel had been using the AHRIS for about two months prior to completing the survey, while the rest of the respondents were able to navigate the system after attending an orientation and demonstration. The orientation and demonstration sessions, which were limited to 20-25 respondents per day, were conducted over the course of a month. Respondents were only allowed to participate in the survey after attending these sessions. The questionnaire, which took approximately 5-10 minutes to complete, was distributed through both online platforms such as Facebook Messenger and in-person at the university clinic. Participants evaluated its acceptability using TAM, emphasizing usefulness, ease of use, intention, and actual usage. Additionally, users' encountered challenges

were documented. The output of the study was the developed AHRIS.

Sampling technique

To guarantee a thorough and representative sample selection procedure, stratified purposive sampling was used. With this approach, the population was categorized into discrete subgroups according to pertinent factors, such as departmental affiliation within each college. From each subgroup, participants were carefully selected to represent both employee and student populations. Specifically, a sample of 101 employees, which included two clinic nurses and 300 students was drawn, ensuring that key groups, such as teaching and non-teaching personnel, contract-based employees, and students from different programs, were adequately represented. This approach allowed the study to capture a diverse and balanced set of perspectives from across the university community.

Statistical analysis

Three statistical techniques were used in the study: Pearson r coefficients, simple percentages, and weighted averages. A five-point Likert scale, with five being the highest level of acceptance and one being the lowest, was used to produce weighted means in order to assess the acceptability of the TAM-based AHRIS. The survey questionnaire underwent content validation by computer technology experts, and reliability testing yielded a Cronbach alpha coefficient of 0.868. Since Cronbach's alpha exceeded the minimum threshold of 0.7, the study inferred that the technical specifications of AHRIS demonstrated strong internal consistency.

Data gathering procedure

The researchers obtained approval from the Campus Director's office at Cebu Technological University, Carmen campus, ensuring ethical compliance (Ref. number: 2024-001-LEC, a unique identifier assigned by the local Ethics Committee representative to confirm the study's adherence to ethical standards). All respondents provided informed consent electronically via Google Forms or in writing. The university administration permitted software demonstrations, ensuring privacy during sessions. The university clinic nurse assisted in data collection and ensured that the AHRIS software used for demonstration complied with all confidentiality and security protocols. Questionnaires were distributed via Google, Facebook Messenger, and face-to-face, depending on respondent availability. Protocols were established for data collection, confidentiality, debriefing, communications, and conflict of interest to ensure ethical standards were upheld throughout the study.

Results

The study involved two main groups of participants: 101 university employees, including clinic nurses, teaching and non-teaching staff, part-time teachers, and contract-based employees, along with 300 students from various programs such as Industrial Technology, Hospitality Management, Marine Engineering, Education, and Fisheries. These participants were selected using stratified purposive sampling to ensure a balanced representation of perspectives across the university community. The clinic personnel had direct access to the system for two months prior to the survey, while the rest of the employees and students interacted with the system following orientation sessions.

The results presented in each table were derived from specific participant groups. Data on the system's technical requirements were gathered from the three computer experts who assessed the AHRIS, while the results on acceptability, challenges, and system usability were obtained from the broader pool of employees and students, reflecting their experiences and interactions with the system.

Table 1 Data on the technical requirements of the Automated Health Records Information System post-exercise, and recovery period

Indicators	Weighted Mean	Description
1. AHRIS design	3.67	very well done
2. AHRIS features	3.50	very well done
3. AHRIS content	3.63	very well done
Overall average	3.60	very well done

A. Technical requirements of the Automated Health Records Information System

As shown in table 1, the result suggested that the technical requirements were considered critical in the development of the university clinic's AHRIS, as indicated by the overall average of 3.60, which was equivalent to a very well done.

Table 1 revealed that the technical requirements of the AHRIS indicated that the AHRIS design, features, and content were all rated very well done, with a mean of 3.67, 3.50, and 3.63, respectively. This result indicated that the system met the highest standards of quality and provided a comprehensive suite of tools and features to support the efficient management of health records.

B. Acceptability of the Automated Health Records Information System

The average score of 4.695, which was similar to a very highly acceptable one, indicated that the system's acceptability was regarded as important in building and implementing the automated health records information system for the university clinic, as shown in table 2.

As indicated by the data depicted in table 2, the TAM served as a beneficial framework for comprehending user acceptance of the AHRIS. The average scores for the acceptability variables were as follows: perceived usefulness (4.78), perceived ease of use (4.60), intention to use (4.71), and actual use (4.69), with an overall average of 4.695. These results suggest a strong level of acceptance of the AHRIS among users.

C. Relationship of the Automated Health Records Information System's acceptability

The findings presented in table 3 of the study indicated that there were no notable correlations observed between the pairs of variables examined. The computed correlation coefficients (r) were all extremely low, ranging from 0.023 to 0.083. Furthermore, every *p*-value was higher than the traditional cutoff point of 0.05, indicating that the null hypothesis—which put out the idea that there were no meaningful intercorrelations between the four variables—could not be ruled out.

D. Challenges encountered in navigating the Automated Health Records Information System

The respondents had varied experiences while navigating the AHRIS. Table 4 presents issues and concerns mentioned by the students, while table 5 mentions the challenges of the employees.

Table 2 Data on the acceptability of the Automated Health Records Information System

Indicators	Weighted Mean	Description
1. Perceived usefulness	4.78	very highly acceptable
2. Perceived ease of use	4.60	very highly acceptable
3. Intention to use	4.71	very highly acceptable
4. Actual use	4.69	very highly acceptable
Overall average	4.695	very highly acceptable

Table 3 Inter-correlation of the acceptability variables

Pair of Variables	Computed r	*p*-value	Decision	Significance
Usefulness and ease of use	0.082	0.101	fail to reject Ho	not significant
Usefulness and intention to use	0.083	0.097	fail to reject Ho	not significant
Usefulness and actual use	0.066	0.187	fail to reject Ho	not significant
Ease of use and intention to use	0.023	0.646	fail to reject Ho	not significant
Ease of use and actual use	0.041	0.413	fail to reject Ho	not significant
Intention to use and actual use	0.041	0.413	fail to reject Ho	not significant

Table 4 The challenges provided by the students

Students' challenges	f	%	Rank
The app is new that is why it takes time for me to fill out the form.	105	35	1
Need to consult the user manual often when using Automated Health Records Information System.	95	31.67	2
Difficult to access the Automated Health Records Information system using a smartphone.	68	22.67	3
Make errors frequently when using the Automated Health Records Information System.	19	6..33	4

Table 5 The challenges provided by the employees

Employee's challenges	f	%	Rank
Need to consult the user manual often when using Automated Health Records Information System.	53	52	1
Need more time to study the app.	34	33	2
Need more in-person training and demonstration about the app to fill in the information correctly.	25	24	3
Cannot view the app with my cell phone.	19	18	4

Discussion

In this section, the study highlighted several critical insights that were pivotal for understanding the adoption, implementation, and use of the AHRIS in a university clinic setting.

Firstly, the technical requirements of the AHRIS, including its system's design, features, and content, received high ratings from experts. These aspects were foundational to the system's functionality, usability, and compliance with regulatory standards. The emphasis on meticulous design ensured that the system met the specific needs of healthcare professionals and patients, contributing to its efficiency and security. The validation of these technical requirements through expert evaluation affirmed the system's capability to manage health information effectively, thereby improving patient care and operational efficiency.

The design was crucial for the AHRIS, demanding careful planning to prioritize security, meet technological standards, and ensure user-friendliness. The design process aligned with

nursing practice, incorporating elements such as quality, safety, security, efficiency, effectiveness, and privacy protocols, while considering the needs of all stakeholders, including administrators, patients, and healthcare professionals[7-10].

To ensure that the program met the requirements of both patients and university clinic staff and contributed to a secure and efficient system, design validation was crucial. Experts recognized potential implementation problems and affirmed that the design effectively addressed healthcare needs. Throughout the design process, important elements such as the user manual, database design, testing, user interface, system architecture, programming language, and interoperability were carefully considered. Detailed flowcharts for the admin and patient portals were developed to visualize the system's capabilities and ensure proper functionality. These flowcharts were crucial in illustrating the processes and interactions within the AHRIS, making the system's workflow clearer for users. The design process began by gathering user requirements from clinic staff and patients through interviews and surveys, which highlighted how each group interacted with the system and identified areas for improvement in terms of efficiency and user-friendliness.

Once these needs were identified, the flowcharts were used to map out the step-by-step processes for both portals. The admin portal's flowchart covered key functions such as patient record management, appointment scheduling, and report generation, ensuring a logical and efficient workflow. For the patient portal, the flowchart focused on user actions such as account creation, appointment requests, viewing medical records, and communication with the clinic. These visual representations played a vital role in reviewing the system's adherence to user requirements, helping experts identify potential bottlenecks, and ensuring the system was user-friendly and aligned with the intended goals.

Features of the program were meticulously crafted to prioritize user-friendliness, ensure clear access to patient health information, and facilitate medical documentation, decision-making, communication among healthcare workers, and data sharing. Expert validation confirmed the system met these technical requirements, enhancing healthcare management within the university clinic. The program streamlined processes for healthcare providers, administrators, and patients, offering functionalities such as generating patient reports, analyzing health data, ensuring secure access to records, and facilitating communication with healthcare professionals. Detailed consultation records provided a structured view of patient interactions and treatments, supporting informed decision-making and optimizing healthcare information management. For adolescents with complicated medical needs and long-term illnesses, the use of electronic health records in schools proved beneficial for managing cases and coordinating care, offering the most efficient, effective, safe, and secure approach to handling student health information[9,17-19].

Content played a crucial role in meeting the technical requirements of the AHRIS, ensuring accurate representation of the system's purpose, accessibility, and user-friendliness. It facilitated data processing and communication between healthcare providers and patients, which was integral to the system's functionality. School nurses effectively accessed student health data stored in EHRs for communication and exchange with other healthcare providers and organizations, enhancing student health outcomes through bidirectional interoperable EHRs[9].

The system was designed, developed, and tested to high standards, ensuring accurate and efficient capture, secure storage, and retrieval of necessary information. Functionalities included user registration and login, management of patient consultation and treatment records, patient history, student enrollment tracking, data management, appointment scheduling, inventory management, and medical report generation. The patient's records, depicted in the system's figures, were a key

component of the content, supporting comprehensive medical histories and treatment plans.

Validation by experts ensured the system met user needs and complied with relevant standards, improving accuracy, reliability, and usability in managing healthcare information within the university clinic environment. In order to assess the quality, usability, functionality, security, and interoperability of school health electronic health records—as well as the associated policy, procedures, and professional development requirements—school nurses needed to be represented on school district information technology committees[19].

Secondly, the study applied the TAM to assess how well users, including healthcare professionals and students, accepted the AHRIS. It found that users generally perceived the system as useful, easy to use, and expressed intention to use it, indicating they viewed it as valuable for enhancing healthcare delivery. The decision of whether to accept or reject the technology was largely influenced by emotions[11,15]. These findings were supported by the study by Davis FD[11], who pointed out that research in information system management had traditionally been motivated by the prospective benefits of technology, influencing people's willingness to adopt new technologies. Thus, easier-to-use technologies perceived as being beneficial were more likely to be adopted, as evidenced by empirical research in the field. In this model, technology that was easier to use was viewed as more advantageous, which encouraged more people to adopt it[11,15].

Despite the lack of significant correlations between the acceptance variables (usefulness, ease of use, intention to use, and actual use), additional contextual factors likely influenced the overall acceptability of the AHRIS. The data did not capture the full range of influences on system adoption. Factors such as institutional support, individual user characteristics, prior experiences, and varying levels of technological skills played significant roles in how users received the AHRIS.

For instance, the orientation sessions and technical support provided by the researchers may have helped less tech-savvy users overcome challenges, increasing actual usage despite initial difficulties. Users with prior experience in digital systems likely had a smoother adoption process. Feedback from tables 4 and 5, where users reported consulting the manual frequently or facing difficulties with mobile access, pointed to varying levels of comfort with technology, reinforcing the impact of these external factors on acceptability.

While the study indicated strong user acceptance of the AHRIS in university clinics, it underscored the importance of considering these external factors for effective system implementation. Understanding how these variables interacted provided insights into optimizing healthcare information systems in academic settings. Future research should further explore these dynamics to enhance system adoption and utilization.

Furthermore, the study underscored significant challenges faced by both students and employees when using the AHRIS in a university clinic. Students encountered issues with software usability, mobile device compatibility, and system reliability. Employees, on the other hand, experienced difficulties such as frequent reliance on user manuals, the time needed to learn the system, and accessing it via smartphones.

Similar difficulties were seen in the study conducted by Press A and colleagues[20], which found that although the electronic tool was first perceived as being time-consuming, difficult to use, and less user-friendly, these issues were resolved after the usability was assessed. Addressing these challenges was crucial for enhancing user acceptance and maximizing the system's effectiveness in supporting patient care and administrative tasks.

This study underscored the importance of ongoing support through comprehensive training sessions and continuous improvements in system usability and compatibility. Proactively addressing

these issues helped institutions improve the adoption and utilization of AHRIS, leading to enhanced healthcare delivery and operational efficiency in university clinics.

Conclusion

The study concluded that technical requirements and software acceptability were critical factors in developing and implementing an automated health records information system (AHRIS). The research emphasized the importance of these technical requirements and software acceptability in successfully deploying the system. Attention to detail in program design, features, and content was essential to meet healthcare professionals' needs and ensure regulatory compliance. Validation by experts confirmed the system's ability to manage health information effectively, leading to improved patient care and operational efficiency.

Although acceptability variables showed no significant interrelationships, other institutional and individual factors, such as institutional support and user characteristics, were important. Adequate training and support during implementation were crucial, as highlighted by respondents. Adoption of the system was essential for university clinics to improve healthcare delivery, patient outcomes, and operational efficiency.

Continuous monitoring and evaluation were vital for identifying and addressing emerging challenges, maintaining data accuracy and security, and achieving the system's intended goals. This iterative approach was essential for adapting to technological advancements and ensuring the system being relevant and effective over time.

The study's findings were particularly relevant for educational institutions seeking to streamline their health records management systems. By prioritizing technical robustness, user acceptance, and ongoing system improvement, stakeholders could optimize the benefits of AHRIS, ultimately improving patient care and organizational efficiency in university clinic environments.

Take home messages

The acceptability of Automated Health Record Information Systems in school settings was driven by their capability to streamline healthcare delivery, enhance data accuracy, and improve patient outcomes through efficient information management.

Conflicts of interest

The authors declare no conflict of interest.

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Concurrent validity of two-dimensional motion analysis using Kinovea for measuring spatiotemporal gait parameters in healthy individuals

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KEYWORDS
Gait; Walking; Psychometric property; Physiotherapy; Rehabilitation.

ABSTRACT

Spatiotemporal gait parameters are usually used as crucial indicators for quantifying rehabilitation effectiveness and several clinical outcomes such as gait and balance ability, and risk of falls of many individuals. Kinovea is an open software for analyzing captured images. The validity of this software has been extensively studied for joint angle measurement, but it is unclear for the measurement of spatiotemporal parameters, which limits its application. Thus, the study explored the concurrent validity and agreement of Kinovea to detect spatiotemporal gait parameters as compared to a criterion measure. Fifty-one healthy participants (age range from 18 to 59 years) were instructed to walk along a 10-m walkway for three trials. Stride length, cadence, gait symmetry and walking speed were assessed using Kinovea and APDM® Mobility Lab (APDM) system. Pearson's correlation coefficients, concordance correlation coefficients (CCC) and Bland–Altman plot were utilized to explore the concurrent validity and agreement of the Kinovea findings and standard measures. Excellent validity and agreement were found for Kinovea in calculating spatiotemporal parameters ($r > 0.95$: $CCC > 0.85$, p-value < 0.001). Moreover, the Bland–Altman plot data were uniformly-scattered around the horizontal axis and under the limit of agreement. The findings conclusively confirm the validity of Kinovea for spatiotemporal parameters as verified using a standard measure. Thus, this software can be used as an alternative assessment for clinicians in various clinical-based and community-based settings.

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Introduction

Spatiotemporal gait parameters, such as stride length, step symmetry and cadence, have been used for various objectives including quantifying rehabilitation effectiveness and being a representative of sensorimotor deficits, walking capability, balance ability, and falls risk[1-4]. Technically, these parameters are delivered via a valid and reliable method of three-dimensional motion analysis or inertial sensors[5,6]. However, the technique has several limitations affecting the application in clinical and community settings, such as high cost, low portability, and the skill needed to operate the systems[7-9]. On the contrary, the conventional footprint has been criticized for its invalid and unreliable for clinical and research works[10].

Consequently, a two-dimensional (2D) motion analysis has been proposed to minimize problems of the aforementioned methods[7-9]. Among many programs of 2D analysis, the Kinovea® (Kinovea) is an open-license software that is commonly used to assess joint angle validly and reliably of several activities such as sitting, running, and jumping[11-14]. However, there are few studies supporting psychometric properties of some spatiotemporal gait measurements such as stride length and gait speed, which limit their clinical and research application for all gait variables needed as clinical outcomes[12,15]. In addition, the existing validity data regarding Kinovea involved only the young adults, whereby a recent study has suggested that gait parameters have been changed throughout lifetime periods[16,17].

Therefore, the objective of this study is to evaluate the validity and agreement of 2D motion analysis using Kinovea software compared to a criterion measure (inertial sensor) in analyzing spatiotemporal parameters during walking at a preferred speed among healthy individuals (age ranged 18 to 59 years). The findings would further support clinical and research utility of Kinovea program for spatiotemporal gait measurements in healthy adults.

Materials and methods

Study design and participants

This study was cross-sectionally carried out between June and August 2023 in healthy adults. The eligible participants aged 18-59 years, had body mass index (BMI) between 18.5–29.9 $kg \bullet m^{-2}$ and were able to understand, communicate, and follow the instructions used in the study, and possessed the ability to walk at least 10 meters without any assistance or devices[2,8]. Individuals with a condition that could affect and confound outcomes during a 10-m walk, such as acute pain or inflammation in the muscles or joints, (i.e., >5 out of 10 on a visual analogue scale), leg length discrepancy (> 3 cm) or lower extremity deformity were excluded from the study[18].

The sample size estimation for the primary objective of the study (concurrent validity) was calculated with the lowest level of correlation (r) reporting in a previous study of 0.45[19] and the α of 0.05. The findings indicated that this study required at least 44 participants to obtain 90% power of test. In addition, systematic review and previous statistical studies recommend that at least 50 participants are adequate to minimize selection bias for a validity and agreement study[20,21]. Thus, this study recruited at least 50 participants and then stratified them into four age groups, including 18-29, 30-39, 40-49, and 50-59 years (at least 10 participants in each group) to ensure the generalizability of the findings in healthy individuals. Eligible participants subsequently signed an informed consent document approved by Srinakharinwirot University Ethics Committee in human research (SWUEC 019/66) before participation in the study.

Research protocol

An assessor was trained by an expert (>5-year experience) for the methods of administering the APDM's Mobility Lab (APDM) and mounting the camera. In details, the camera was mounted on a stationary tripod in the middle of the walkway at approximately 3.5 meters away from and

perpendicular to the walkway to capture gait variables of participants over the middle 6-m of a 10-m walkway (at least three-consecutive stride lengths)[22] (Figure 1). Then a preliminary study was conducted in ten participants to assure smoothness of the protocol.

The eligible participants were interviewed and assessed for their demographics, i.e., age, gender, BMI, and vital signs. Three opal wearable sensors of APDM were attached at the base of the 5th lumbar spine and centered on top of the left and right feet of the participant (Figure 1)[5,23].

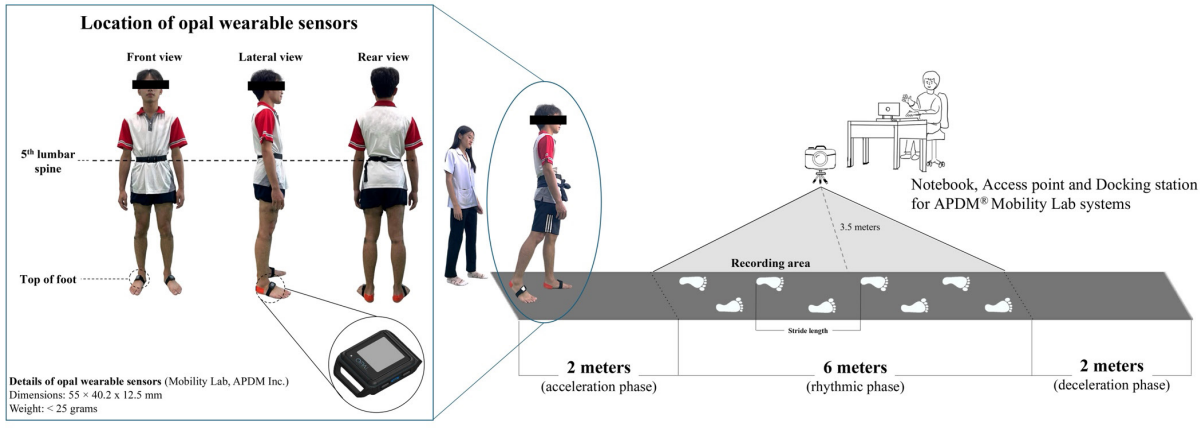

Figure 1 Testing protocol and setting of data recording.

Testing protocol

Participants were instructed to walk barefoot along a 10-meter walkway for three trials at their preferred speed. During the test, their three-consecutive stride lengths of each lower extremity were recorded over six meters in the middle of the walkway to obtain the data over a rhythmic phase (Figure 1). Participants were fastened with a lightweight safety belt with an assessor always being or walking alongside them. The participants could take a period of rest (if needed)[18].

After the test, the spatial data were then analyzed from 2D motion video using a method of manual digitization in Kinovea by counting the pixels between the distances from the heel of one leg to the heel of the other leg and scaling with respect to the calibration object. Then, the average step length of each lower extremity was subsequently calculated using the following formula[22].

$$\text{Stride length} = \text{average right step length} + \text{average left step length}$$

$$\text{Percent of gait symmetry} = \left[\frac{\text{(average data of the shorter stride length)}}{\text{(average data of the longer stride length)}} \right] \times 100$$

$$\text{Walking speed} = (\text{stride length} \times \text{cadence}) \times 120^{-1}$$

In addition, cadence was obtained by calculating the number of steps in a minute.

$$\text{Cadence} = \text{number of steps in one minute}$$

Data acquisition and processing

Instrumentations

Criterion measures: Previous studies reported that inertial wearable sensors can be used as substitution for conventional laboratory gait analysis systems[5,23,24]. Thus, APDM's Mobility Lab™ (APDM Inc., Portland, OR, USA) was selected as the gold standard gait measurement tool in the current study[23]. Spatiotemporal gait parameters were calculated using an algorithm in a gyroscope (±400◦/s range) and an accelerometer (±5g range) with a sampling rate of 200 Hz to record angular movement and acceleration[23] (Figure 1).

2D motion analysis: A high-resolution (1,920 x 1,080 pixel of the full HD standard) Panasonic Lumix DC-G95 camera (Panasonic Corporation Co., Ltd., Osaka, Japan) was used to collect data during 10-m walkway. Prior to the test, the algorithm of Kinovea software (Kinovea 0.9.5 for Windows; available at http://www.kinovea.org) was calibrated using the known length object (33.5 x 45.5 x 10 cm) with an obvious marker at the corner of the box[8,22].

Statistical analysis

Descriptive statistics were used to explain the demographics of participants as well as the findings of the study. The Kolmogorov-Smirnov test was applied to estimate the normality of the data distribution. Pearson's correlation coefficient (r) was used to explain the levels of correlation (concurrent validity) and relative agreement between the two systems. The strength of the correlation was defined as poor (r = 0.30–0.49), moderate (r = 0.50–0.69), and excellent (r > 0.70)[20]. The levels of significant differences were set at *p*-value < 0.05[25].

To measure agreement, Bland-Altman analysis was used to inspect the error scores between the two systems with respect to the mean scores. This method demonstrates any tendency for the variation to change with the magnitude of the measurement[26]. Moreover, the concordance correlation coefficient (CCC) was applied to obtain an overall agreement between the two systems. The level of the CCC was determined as poor (CCC < 0.4), moderate (CCC = 0.40–0.74), and excellent (CCC > 0.74)[27]. In addition, the percentage error was computed to express the standard deviation (SD) of the between-method difference scores (bias) as a percentage of the mean measure of the two systems using the formula[7]:

$$PE = (100 \times (2SD \text{ of bias})/[(Mean_{Kinovea} + Mean_{APDM})/2])$$

Results

A total of 54 individuals were screened for their eligibility according to the criteria of this study, and three individuals were excluded because of BMI exceeding normal range (n=2), and signs of inflammation of the lower extremity (n=1). The 51 remaining participants were enrolled and completed the study. Their average age was approximately 38 years old with a normal BMI and most of them were female. Stride length, gait symmetry, cadence, and walking speed are also summarized in table 1.

The percentage error and agreement values are provided in table 2. Stride length, gait symmetry, cadence, and gait speed possessed strongly excellent correlations and overall agreement between both systems, with low percentage errors (r and CCC values > 0.85 and percentage error = 2–5%). The linearity assessment for the agreement of landmark detection with respect to distance from Kinovea software is provided in figure 3, with the points on the Bland–Altman plot being uniformly and tightly scattered around the horizontal axis (Figure 3).

The correlations between 2D motion analysis using Kinovea and APDM systems were also high for the stride length, gait symmetry, cadence, and gait speed (r = 0.91 - 0.99, *p*-value < 0.01) (Figure 2).

Table 1 Demographic of participants

Variable	N = 51	Min/Max
Number of participants in each age group: n (%)		
- 18-29 years	14 (27.45)	-
- 30-39 years	15 (29.41)	-
- 40-49 years	11 (21.57)	-
- 50-59 years	11 (21.57)	-
Gender: Female: n (%)	33 (64.70)	-
Age[a] (years)	37.73 ± 11.84 (34.40-41.06)	18/59
Weight[a] (kg)	60.70 ± 10.19 (57.83-63.56)	43/79
Height[a] (cm)	160.69 ± 7.81 (158.49-162.88)	145/175
Body mass index[a] ($kg \bullet m^{-2}$)	23.42 ± 3.06 (22.56-24.28)	18.7/29.7
Stride length[a] (meter)	1.20 ± 0.11 (1.18-1.21)	0.98/1.50
Gait symmetry (%)	97.72 ± 2.28 (97.36-98.08)	90/99
Cadence[a] (step/minute)	119.97 ± 8.72 (118.58-121.37)	100/144
Gait speed[a] (meter/second)	1.21 ± 0.17 (1.18-1.23)	0.85/1.7

Note: [a] The data are presented as mean ± standard deviation (95%CI).

Table 2 Mean difference in 2D motion analysis (Kinovea) and inertial sensors (APDM) system derived spatiotemporal gait parameters, along with 95% limits of agreement (LoA), percentage error (PE), and concordance correlation coefficients (CCC) (n = 51).

Variable	$Mean_{APDM}$	$Mean_{Kinovea}$	Mean diff[a]	95% LoA	PE[b] (%)	CCC (95% CI)
Stride length (meter)	1.20 ± 0.11	1.25 ± 0.11	0.05	-0.01 to 0.11	5	0.86 (0.83 to 0.89)*
Gait symmetry (%)	97.72 ± 2.28	98.12 ± 2.02	0.40	-1.54 to 2.34	2	0.89 (0.86 to 0.93)*
Cadence (step/minute)	119.97 ± 8.72	119.99 ± 8.85	0.02	-2.47 to 2.49	2	0.99 (0.98 to 0.99)*
Gait speed (meter/second)	1.21 ± 0.17	1.28 ± 0.17	0.07	0.01 to 0.15	5	0.88 (0.85 to 0.91)*

Note: [a] Mean diff: mean difference.

[a] Mean differences were derived from the formula as $Mean_{Kinovea}$ - $Mean_{APDM}$.

[b] Percentage error was calculated as (100 x (2SD of bias)/ (($Mean_{Kinovea}$ + $Mean_{APDM}$)/2)).

* *p*-value < 0.001 from the concordance correlation coefficient.

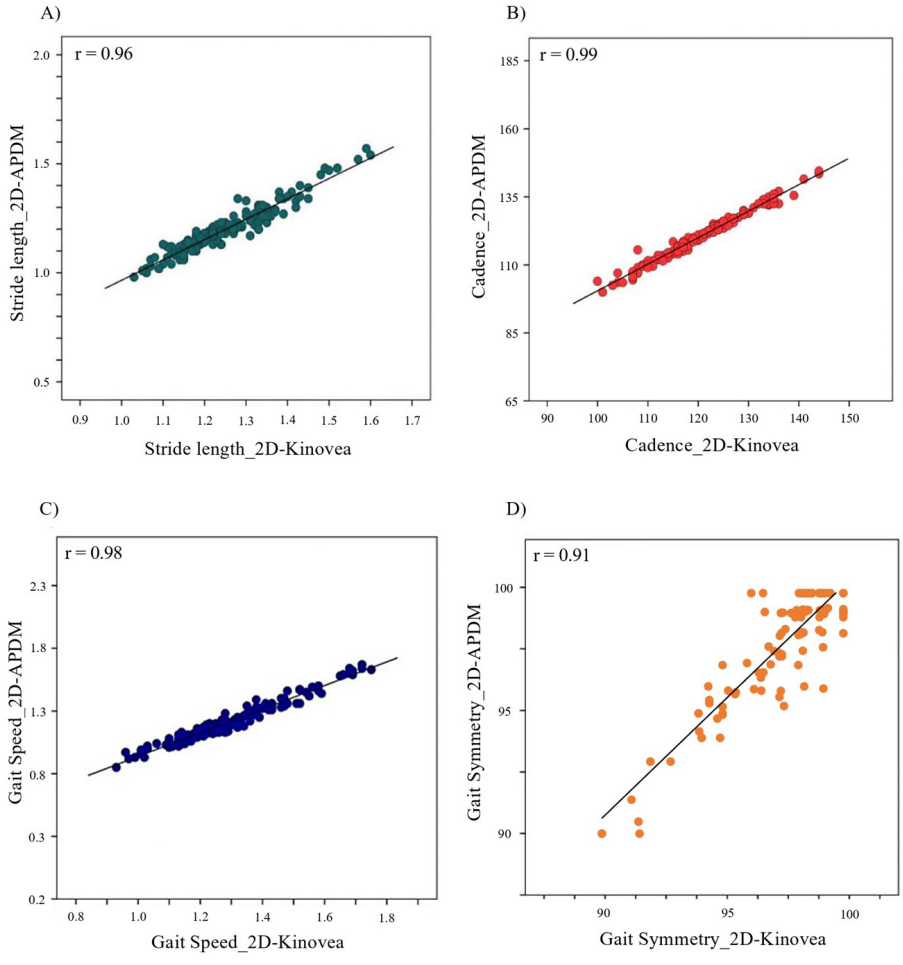

Figure 2 The correlation of spatiotemporal variables from 2D motion analysis (Kinovea) and inertial sensors (APDM) system using the Pearson's correlation (r) analysis.
(A) Stride length
(B) Cadence
(C) Gait speed
(D) Gait symmetry

Abbreviations: 2D-Kinovea, two-dimensional motion analysis using Kinovea; APDM, Inertial opal wearable sensor system.

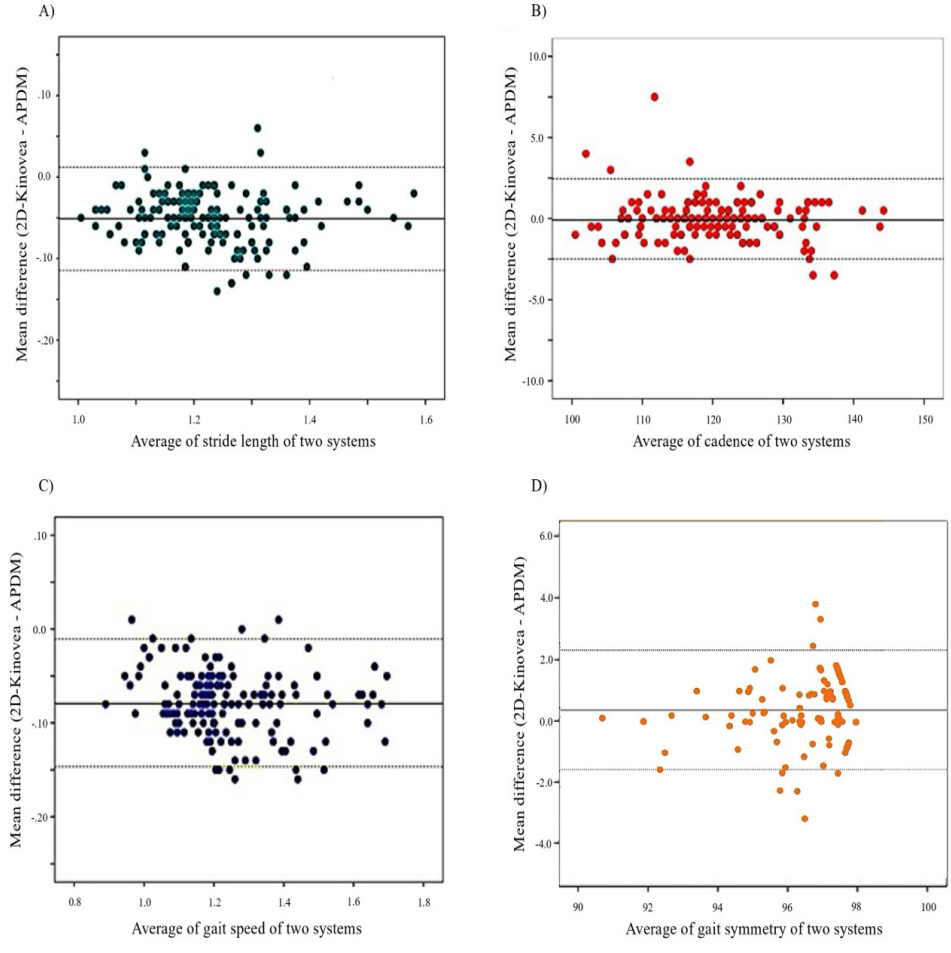

Figure 3 Bland-Altman plots comparing spatiotemporal parameters between 2D motion analysis (Kinovea) and inertial sensors (APDM) system.
(A) Stride length
(B) Cadence
(C) Gait speed
(D) Gait symmetry

Abbreviations: 2D-Kinovea, two-dimensional motion analysis using Kinovea; APDM, Inertial opal wearable sensor system.

Discussion

Laboratory systems for spatiotemporal gait measurements are sophisticated, and thus they are costly and require experienced assessors, which limit their application to support current healthcare paradigm to distribute service to various settings[5,6]. Thus, recent research has been focused on validation and accuracy the psychometric properties of 2D motion analysis to estimate gait parameters among several software[8,9,14]. However, the existing evidence that reported the validity and accuracy of Kinovea in measuring spatiotemporal gait parameters has been limited[15]. The current study found that the 2D-motion analysis using Kinovea is a valid and has acceptable interchangeability with inertial

sensor used in the current study (APDM) to assess spatiotemporal gait parameters, including stride length, gait symmetry, cadence, and gait speed during walking at a comfortable speed in healthy adults.

This study illustrated that Kinovea had extremely high correlation (r = 0.91 – 0.99; *p*-value < 0.01) and high acceptable agreement (CCC = 0.86 – 0.99; PE < 5 %) to a criterion measure (APDM) for estimating gait parameters[7,19,27]. These findings provide clear data regarding the properties of Kinovea to measure spatiotemporal parameters that can be used as an alternative and surrogate method for spatiotemporal parameter measurement. A previous study examined the measurement properties between the 2D motion system using a 1-camera method and a gold standard 3D motion system, namely VICON[28]. They reported that the two systems had significantly different accuracies that were dissimilar compared to the current finding. However, one study examined the validity and agreement of SGAS software, which was in high agreement as compared to a criterion measure close to what we observed[8].

However, the present study found that Kinovea has a lower agreement in spatial parameters (CCC= 0.86-0.89) than temporal variables. A plausible explanation for these findings may be due to a number of software and hardware factors, including (1) the irregular sampling rate of Kinovea, which typically is set at 30 frames/second, and (2) distortion in a simple lens possibly affecting the image shape, especially at the border, which could alter the appearance and perspective of video shots[29]. These factors may interfere with the pixel of the images, which could additionally affect the manual digitization process used to detect the spatial data of this study. Although the psychometric properties of 2D motion system using Kinovea to detect a stride length were lower than the other variables, they were still in an acceptable range for clinical and research application.

The current findings confirmed the use of Kinovea as a proxy 2D-spatiotemporal gait assessment among healthy adults. With its simplicity, the findings support the use of Kinovea to promote a standard screening and monitoring of gait parameter in various settings. However, there are some limitations in this study. The current findings did not cover all important spatial variables, especially a step length because the APDM's Mobility Lab system does not offer these data[30]. In addition, participants included only healthy adults, aged 18 to 59 years. As the gait variables are altered throughout lifespan and abnormality, a further study should explore clinical application of Kinovea covering all age groups, as well as those with pathologies affecting their gait manners.

Conclusion

2D motion analysis using Kinovea is a valid and accurate system for assessing stride length, cadence, and gait speed while walking at a preferred speed of healthy adults. With its practicality, the present findings support the application of 2D gait assessments using Kinovea to facilitate standard screening with periodic monitoring for gait alteration in various clinical and research settings.

Take home messages

2D gait analysis using Kinovea program provides valid and reliable spatiotemporal outcomes. As a simple and practical method, this system can be used to enhance standard screening with periodic follow-up for gait manners in various clinical and research settings.

Conflicts of interest

The authors declare no conflict of interest.

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ORIGINAL ARTICLE

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Ability of five times sit-to-stand to determine functional limitation in people with knee osteoarthritis

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KEYWORDS
Sit-to-stand;
Clinical measure;
Muscle strength;
Mobility;
Joint arthritis.

ABSTRACT

Knee osteoarthritis (KOA), the most common form of arthritis, affects the ability to fully use the lower extremities due to pain, muscle weakness, and deformity. With the ability of the five times sit-to-stand test (FTSST) to reflect functional lower limb strength, the researchers hypothesized that outcomes of the test could detect functional limitation in these individuals. Thus, this study explored the ability of the FTSST to determine functional limitation in individuals with KOA. Ninety-three participants with KOA were cross-sectionally assessed for their health status, KOA information, FTSST, and functional limitation using a standard measure, namely the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) questionnaire. The findings indicated that 64 participants were those with non-functional limitation, and the rest of them (n = 29) were those with functional limitation according to the data of WOMAC. Furthermore, outcomes of the FTSST ≥ 15 seconds could excellently determine those with functional limitation (sensitivity=79%, specificity=71%, and area under the curve=0.838). As being a practical measure of the FTSST, the present findings offer an alternative strategy to detect and monitor individuals with KOA who have functional limitation, as well as indicate treatment effectiveness for these individuals.

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Introduction

Knee osteoarthritis (KOA) is a progressive degenerative joint disease that is caused by degeneration of articular cartilage and changes in subchondral bone[1]. Major clinical signs and symptoms of KOA include chronic joint pain, morning stiffness over a short period, limited range of motion, crepitus, tenderness, joint effusion, malalignment or deformity of the knee joint, and bony enlargement[2,3]. These consequences further affect muscular functions around the joints, induce mobility restriction, disability, as well as quality of life of the individuals[4-11]. Therefore, the ability of early detection for functional limitations among individuals with KOA is crucial for the initiation of appropriate treatments and monitoring for treatment effectiveness.

Currently, clinical measurements for functional limitation in people with KOA are executed using a test battery, namely the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC)[12,13]. Such test batteries commonly have low sensitivity to detect change and are time-consuming to complete all items in the test, i.e., approximately 10 to 15 minutes[13]. Therefore, the use of a practical measure relating to the impairments due to KOA may offer an alternative strategy to detect a crucial consequence, i.e., functional limitation, among these individuals.
From literature review, the researchers found the five times sit-to-stand test (FTSST) which is commonly used to assess functional lower extremity motor strength and balance ability while changing postures[14,15]. Previously, some studies have reported the use of FTSST to detect problems of individuals with KOA[16-18]. Master et al[17] reported that the ability to complete FTSST of longer than 12 seconds could determine inadequate ability to walk at least 6,000 steps/day in people with KOA (with 80%-95% specificity). Another study also indicated that the time to complete FTSST of more than 7.9 seconds could detect fall risk probability in these individuals[18]. However, to the best of the researchers' knowledge, there is no target value indicating functional limitation, a common consequence of KOA, using the FTSST. Thus, this study investigated the possibility of using the FTSST to determine functional limitation in people with KOA. The findings would offer specific target value indicating functional limitation using practical measure that can be applied for early screening with periodic follow-up of these people in various settings.

Materials and methods

Study design and participants

This cross-sectional study was conducted in people with KOA from several communities in Northeast and southern areas of Thailand. The sample size was estimated using the data from 30 pilot cases with the sensitivity of FTSST being 83%, while the prevalence of Thai frailty people with KOA was around 69.1%[19]. The α error and the precision of the estimation were set at 0.05 and 10% (0.1), respectively. Thus, this study required at least 77 participants. The research protocol of this study was approved by the Khon Kean University Ethics Committee for Human Research (HE652028). Participants needed to sign an inform consent document prior to the participation in the study.

The inclusion criteria were aged between 50 and 79 years, both males and females with unilateral or bilateral KOA as determined using the criteria from the American College of Rheumatology (ACR) classification[20]. The ACR's criteria consists of having knee pain (≤ 7 out of 10), with the addition of 3 out of 6 criteria, including the age of 50 years and over, crepitus on motion, morning stiffness, bony enlargement, bony tenderness, and no palpable warmth[21]. Moreover, the eligible participants needed the ability to perform the test, and understand the command and protocol of the study[22,23]. These individuals were excluded if they had a history of lower extremity joint replacement surgery, with any neurological impairments and residual defect involving mobility, as well as any signs and symptoms affecting the ability to complete the test[16,22,24].

Research protocol

The eligible participants were assessed for their functional limitation using WOMAC questionnaire and FTSST. Details of the tests are as follows.

WOMAC questionnaire

This questionnaire contains 24 items, which are divided into three subscales, including pain (5 items), stiffness (2 items), and physical function (17 items), with four scales of each item. Thus, the maximum WOMAC score is 96. Among these items, the level of functional limitation was considered using the physical function subscale. Participants with the score at least 28 out of 68 were classified as having functional limitation[13].

FTSST

Participants were asked to cross their arms on their chest and sat with their back against the backrest of the chair (43 cm chair height). The participants were instructed to stand up and sit down for five times as quickly as they could do safely. Timing began with the command "Go" and stopped when the participant's back touched the chair on the fifth repetition. They were asked to perform three trials; then the average time was recorded in seconds[25].

Before the tests, participants were asked to wear sandal shoes and safety belt for their safety prepared by the researchers. A researcher was at their side without interruption to provide the assistance as needed and accuracy of the outcomes. Participants were able to take a period of rest between the trials as needed until their vital signs returned to their usual data.

Statistical analysis

The data were analyzed using the SPSS for Windows, version 22.0 (SPSS Statistics version 17.0; IBM Corporation, Armonk, NY, USA; serial number: 5068054). The descriptive statistics (mean, standard deviation, and percent) were applied to explain demographic characteristics of the participants. If the data were normally distributed, the independent samples t-test and Chi-square test were used to compare the findings between the groups (non-functional limitation and functional limitation groups) for continuous and categorical variables, respectively. However, if the data were non-normally distributed, the Mann-Whitney U Test and Fisher's Exact test were applied for the same objectives. Then, the Receiver Operator Characteristic (ROC) curve was applied to determine an optimal sensitivity and specificity for functional limitation of the participants. A level of significant difference was set at less than 0.05[26].

Results

Demographic characteristics of the participants

One hundred and thirty-two individuals with KOA were interested to participate the study. However, 26 individuals were excluded because of missing the inclusion criteria (Figure 1). Thus, a total of 106 participants completed the study, and were divided into non-functional limitation (n = 68, 64.1% and functional limitation (n = 38, 35.9%) groups based on their WOMAC (functional part) scores. All demographic characteristics of the participants were shown in table 1. However, 13 participants were unable to perform FTSST due to exhaustion, increased knee pain, and requiring upper limb contribution for standing up. Thus, 93 participants were able to complete the FTSST.

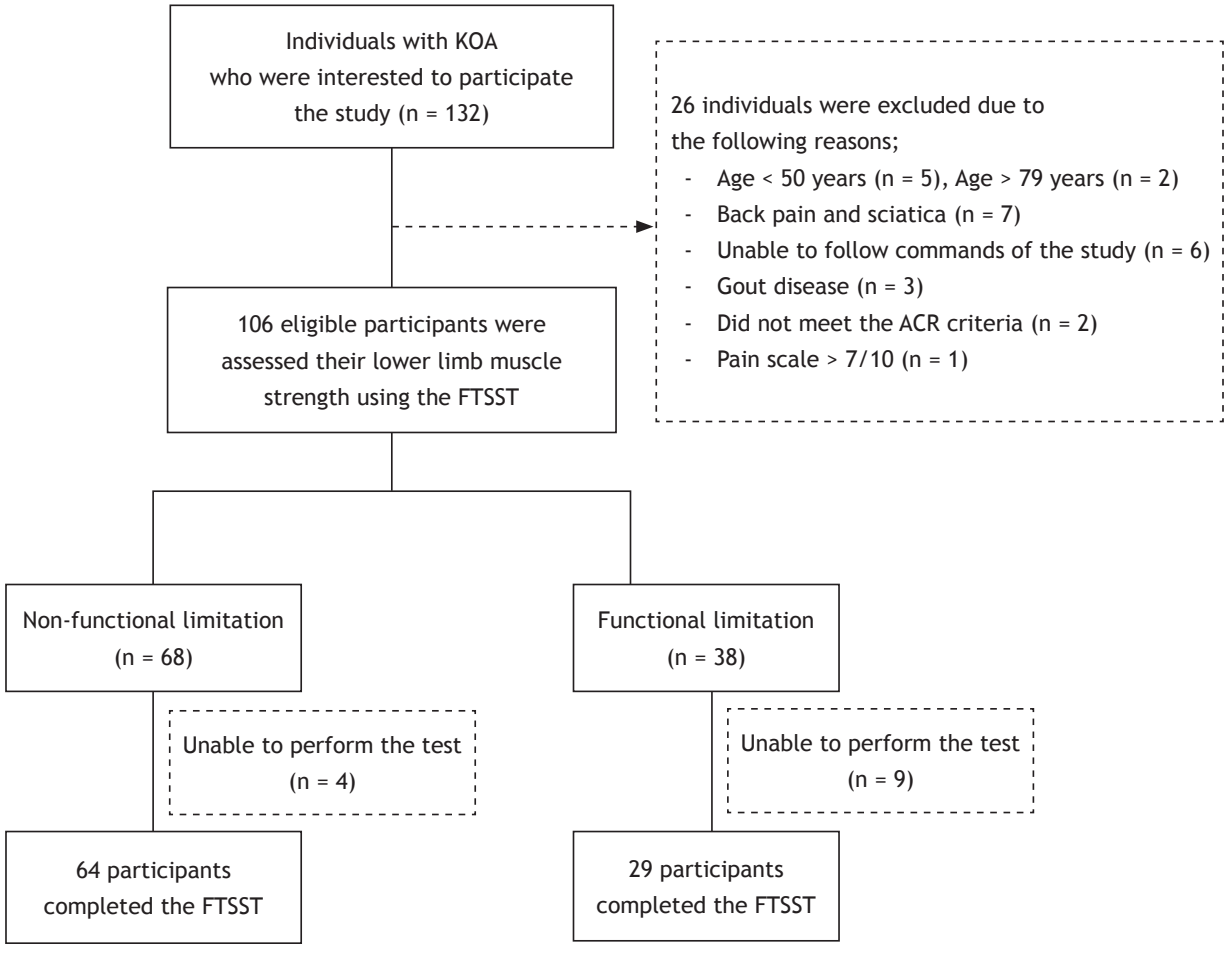

Figure 1 Participation flowchart.
Abbreviations: KOA, knee osteoarthritis; ACR, American College of Rheumatology; FTSST, five times sit-to-stand test.

FTSST data

Table 2 presents the FTSST data. All participants could complete the FTSST in approximately 15s, whereby those with functional limitation group used time to complete the test significantly longer than those in the non-functional limitation group (*p*-value < 0.001). The duration to complete the FTSST 15s and over could excellently determine those with functional limitation (Table 3).

Table 1 Demographic characteristics of the participants

Variable	All (n = 106)			Non-functional limitation (n = 68)			Functional limitation (n = 38)			*p*-value
	Mean ± SD (95%CI)	Median	Min - Max	Mean ± SD (95%CI)	Median	Min - Max	Mean ± SD (95%CI)	Median	Min - Max	
Sex: female, n(%)[†]	83 (78.3)			47 (69.1)			36 (94.7)			0.002[a,*]
Age (years)	61.77 ± 8.04 (60.33 – 63.35)	61	50 – 79	25.60 ± 4.07 (24.62 – 26.59)	59	50 – 79	26.69 ± 4.75 (25.13 – 28.25)	65.5	50 – 79	<0.001[d,*]
BMI (kg/m^2)	25.99 ± 4.33 (25.16 – 26.83)	25.64	16.23 – 37.38	3.87 ± 1.23 (3.57 – 4.17)	25.11	17.22 – 37.38	5.16 ± 1.08 (4.80 – 5.51)	26.33	16.23 – 35.00	0.21[6c]
NRS (score)	4.33 ± 1.33 (4.08 – 4.59)	4	2 – 7		4	2 – 6		5	2 – 7	<0.001[d,*]
ACR items										
Average ACR score	5.19 ± 0.83 (5.04 – 5.36)	5	3 – 6	4.99 ± 0.85 (4.78 – 5.18)	5	3 – 6	5.58 ± 0.64 (5.35 – 5.77)	6	5 – 6	<0.001[d,*]
ACR classification, n(%)[†]										<0.001[b,*]
Age ≥ 50 years	106 (100)			68 (100)			38 (100)			
Morning stiffness	97 (91.5)			59 (86.8)			38 (100)			
Crepitus	91 (85.8)			54 (79.4)			37 (97.4)			
Bony tenderness	101 (95.3)			64 (94.1)			37 (97.4)			
Bony enlargement	56 (52.8)			27 (39.7)			29 (76.3)			
No palpable warmth	106 (100)			68 (100)			38 (100)			
Type of KOA lesion, n(%)[†]										
Unilateral : Bilateral	52 (49.1) : 54 (50.9)			38 (55.9) : 30 (44.1)			14 (36.8) : 24 (63.2)			0.060[a]
Knee deformity [Yes, n(%)][†]	43 (40.6)			21 (30.9)			22 (57.9)			0.007[a,*]
WOMAC Index (score)	31.43 ± 16.78 (28.12 – 34.74)	28.50	7 – 87	21.50 ± 9.00 (19.36 – 23.66)	21	7 – 41	49.21 ± 12.12 (45.51 – 53.06)	44.5	36 – 87	<0.001[d,*]
WOMAC (Pain)	5.74 ± 3.25 (4.08 – 4.59)	5	1 – 20	4.12 ± 1.99 (3.62 – 4.61)	4	1 – 10	8.63 ± 3.07 (7.74 – 9.71)	8	2 – 20	<0.001[d,*]
WOMAC (Stiffness)	2.52 ± 1.35 (2.28 – 2.76)	2	0 – 8	2.22 ± 1.26 (12.95 – 2.54)	2	0 – 8	3.05 ± 1.37 (2.66 – 3.52)	2	2 – 8	0.002[c,*]
WOMAC (Functional)	23.15 ± 13.12 (20.58 – 25.74)	21	5 – 59	15.18 ± 6.82 (13.54 – 16.80)	15	5 – 28	37.42 ± 8.87 (34.62 – 40.07)	34	29 – 59	<0.001[d,*]

Note: The data were compared using independent t-test, *Indicated significant difference (*p*-value < 0.001).

Abbreviations: FTSST, five times sit-to-stand test; SD, standard deviation; CI, confidence interval.

Table 1 Demographic characteristics of the participants (Cont.)

Variable	All (n = 106)			Non-functional limitation (n = 68)			Functional limitation (n = 38)			*p*-value
	Mean ± SD (95%CI)	Median	Min - Max	Mean ± SD (95%CI)	Median	Min - Max	Mean ± SD (95%CI)	Median	Min - Max	
KOA duration (month)	39.88 ± 45.77 (31.05 – 49.35)	24	1- 228	29.82 ± 39.53 (21.55 – 40.02)	12	1 – 228	57.88 ± 50.95 (42.94 – 73.06)	36	4 – 216	<0.001[d,*]
Underlying disease [Yes, n(%)][†]		70 (66)			44 (64.7)			26 (68.4)		0.699[a]
Level of education, n(%)[†]										<0.001[b,*]
Under elementary school		14 (13.2)			4 (5.9)			10 (26.3)		
Elementary school		65 (61.3)			39 (57.4)			26 (68.4)		
Secondary school		11 (10.4)			9 (13.2)			2 (5.3)		
College		16 (15.1)			16 (23.5)			0 (0)		

Note: [†] The data are presented using the number (percent of total participants). [a] Data were compared using Chi-square test, [b] Fisher's Exact test, [c] Independent samples t-test, and [d] Mann-Whitney U Test, * Indicated significant differences (*p*-value < 0.05).

Abbreviations: BMI, body mass index; NRS, numeric rating scale; ACR, American College of Rheumatology; KOA, knee osteoarthritis; WOMAC, Western Ontario and McMaster Universities Osteoarthritis Index.

Table 2 Comparison of the FTSST between non-functional limitation and functional limitation groups

Test	All (n = 93)		Non-functional limitation (n = 64)		Functional limitation (n = 29)		Mean difference (95%CI)	*p*-value
	Mean ± SD (95%CI)	Min - Max	Mean ± SD (95%CI)	Min - Max	Mean ± SD (95%CI)	Min - Max		
FTSST (s)	15.35 ± 4.95 (14.36 – 16.44)	8.93 – 33.49	13.35 ± 2.63 (12.71 – 13.99)	8.93 – 19.82	19.77 ± 5.96 (17.59 – 21.97)	10.05 – 33.49	6.42 (4.66 – 8.19)	<0.001*

Note: The data were compared using independent t-test, *Indicated significant difference (*p*-value < 0.001).

Abbreviations: FTSST, five times sit-to-stand test; SD, standard deviation; CI, confidence interval.

Table 3 Cut-off scores of FTSST to determine functional limitation

Test	n	Cut-off score	Sensitivity (%)	Specificity (%)	AUC	95%CI of AUC
FTSST (s)	93	≥ 15.00	0.79	0.71	0.838	0.74 – 0.93

Abbreviations: FTSST, five times sit-to-stand test; AUC, area under the curve; CI, confidence interval.

Discussion

The study compared the FTSST data between participants with functional limitation and non-functional limitation. In addition, the study explored the specific cut-off score of the FTSST to indicate functional limitation in people with KOA. The findings indicated that approximately one-third of the participants with KOA had functional limitation. Participants with functional limitation used the time to complete the FTSST significantly longer than those with non-functional limitation (Table 2). The FTSST ≥ 15 seconds could excellently indicate functional limitation in people with KOA (Table 3).

To the best of the researchers' knowledge, there were no data on functional limitation in individuals with KOA available. The current findings indicated that more than one-third (35.9%) of participants with KOA experienced functional limitation. These individuals had significantly poorer ability to perform FTSST as compared to those without functional limitation (*p*-value < 0.001). These findings may reflect their natural characteristics, whereby they were older with significantly greater number of females and those with knee deformity (*p*-value < 0.01, table 1). In addition, they had significantly longer duration after having KOA, higher pain severity with low level of education as compared to those without functional limitation (*p*-value < 0.01, table 1). These findings associated with previous reports that individual factors including severity of KOA, knee deformity, level of pain and joint stiffness, and a long duration after KOA significantly induced functional limitation[27,28]. Having such characteristics crucially affect the ability to complete the FTSST. Eriksrud et al[29] reported that, apart from lower-extremity range of motion and balance ability, the FTSST is crucially affected by lower-extremity muscle force. Wretenberg and Aborelius[30] found that the knee extensors contribute 72% of the concentric force at the hip and the knee joint while completing a sit-to-stand task. As a demanding activity for lower limb muscles and motion, participants with functional limitation who had greater pain severity and the higher number of those with knee deformity completed the FTSST using significantly longer duration than those with non-functional limitation (Table 2). Furthermore, the high demanding limited the ability of 13 participants with KOA, regardless of functional limitation to complete the test (Figure 1).

The findings further suggested that the ability to complete the FTSST ≥ 15 seconds (79% sensitivity, 71% specificity) could indicate functional limitation in people with KOA (Table 3), which was longer than that of the another event in individuals with KOA[18]. Amano and Suzuki[18] found that the time to complete the FTSST of greater than 7.9 seconds (92% sensitivity, 58% specificity, and AUC=0.79) could detect the possibility of fall in people with KOA[18]. Although they applied the same protocol of FTSST of the present study, KOA condition was diagnosed using the radiography. Even though the radiography is a standard method, the participants with asymptomatic KOA without any pain were included into their study. Therefore, using the ACR criteria to diagnose KOA condition help to recruit people with symptomatic KOA, who are more likely to have functional limitation. Additionally, the elderly women who completed the FTSST more than 13 seconds (85.7% sensitivity and 53.2% specificity, and AUC=0.72) indicated the highest probability to have sarcopenia[31]. They included the participants focusing on muscle strength and performance. The findings from previous report may imply that although KOA participants in the present study (mean score of FTSST as 13.35 ± 2.63 seconds) had no functional limitation, they might have a chance to develop poor performance and sarcopenia.

The present findings offer evidence supporting the use of a practical and demanding measure, namely the FTSST, to screen and monitor functional limitation of individuals with KOA. The present study applied the FTSST because it is a test specifically demanding for the knees. Therefore,

clinical application of the test should be used with caution as it might aggravate pain severity of the individuals. A further study may apply other indirect practical measures relating to KOA to offer practical measure to be used in all individuals with KOA.

Conclusion

The study compared the outcomes of FTSST between participants with KOA who had functional limitation and non-functional limitation, and explored the specific cut-off score of the FTSST to indicate functional limitation in these individuals. The findings indicated that approximately one-third of the participants with KOA had functional limitation, whereby they could complete the FTSST significantly longer than those with non-functional limitation. The FTSST ≥ 15 seconds could excellently indicate functional limitation in people with KOA.The findings suggest the use of FTSST as a practical and demanding measure to screen and monitor functional limitation of individuals with KOA.

Take home messages

More than one-third of people with KOA had functional limitations. The use of FTSST and its target value of ≥ 15 seconds might help to screen and monitor individuals with KOA to minimize the possibility of having functional limitation in these individuals.

Conflicts of interest

The authors declare no conflict of interest.

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ORIGINAL ARTICLE

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Inhibitory effects of methanolic extracts of *Shorea roxburghii* flowers on gastrointestinal cancer and their antioxidant properties

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KEYWORDS
Natural plant;
Phenolic;
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Antioxidant;
Anticancer.

ABSTRACT

Natural plants contain distinctive secondary metabolites valuable in developing functional foods, nutraceuticals, and pharmaceuticals for preventing and treating various diseases. Many parts of *Shorea roxburghii*, a Southeast Asian plant, contain various bioactive compounds, which have garnered attention for their medicinal properties. This study aimed to extract *S. roxburghii* flowers using methanol as a solvent and to assess their phenolic and flavonoid contents, as well as their antioxidant activity through FRAP and DPPH assays. Additionally, the potential anticancer activities on gastrointestinal cancer were investigated using cytotoxicity and apoptosis assays. The flower extract's phenolic content was determined to be 161.20 ± 0.66 µg GAE/mg, with a flavonoid content of 51.93 ± 5.16 µg QE/mg. The extract exhibited moderate antioxidant activity with 57.31 ± 4.29 µg AAE/mg of FRAP value and an EC50 value of 528.10 µg/ml for DPPH scavenging activity. The methanolic extract of *S. roxburghii* showed cytotoxic effects on AGS and KKU-100 gastrointestinal cancer cell lines, with IC50 values of 57.81 µg/ml and 122.5 µg/ml, respectively. Methanolic extract inhibited cell proliferation by inducing apoptosis, suggesting its potential as an anti-cancer agent. This study highlights the medicinal potential of *S. roxburghii* flowers, emphasizing their rich phenolic content and significant antioxidant and anti-cancer properties.

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Introduction

Reactive oxygen species (ROS) are generated in cellular metabolic processes, and regulated by endogenous antioxidant mechanisms[1]. Imbalance between ROS generation and antioxidant defense mechanisms can cause oxidative stress, resulting in cellular damage and contributing to the pathogenesis of various diseases such as cancer[2]. Recent epidemiological data revealed that gastrointestinal cancers constitute over 25% of all cancer diagnoses and about 33% of cancer-related deaths worldwide. Notably, regions in East Asia demonstrate a high prevalence of gastrointestinal cancer, including gastric and liver cancers. This has a strong association with factors such as the widespread incidence of *Helicobacter pylori* and liver fluke infection within these areas[3]. Pathogen infection causing chronic inflammation with elevated levels of ROS, resulting in damage to biomolecules, initiation of mutations, and eventually leading to the development of cancer[4].

Phytochemicals, which are secondary metabolites derived from plants, have been used directly or chemically modified to create compounds used in current medicine. Flavonoids are the most well-studied subgroup of phenolic compounds, which are the largest group of phytochemicals. They are recognized for their critical functions in plant biology and extensive health benefits. The compounds contain an aromatic ring structure with one or more hydroxyl groups, giving them antioxidant activity, reduction potential, hydrogen donation capacity, and singlet oxygen quenching ability[5]. The antioxidative properties inherent in these phytochemicals play a role in protecting biomolecules and cellular structures from oxidative damage. Medicinal plants and bioactive compounds derived from them provide a novel strategy for treating cancer. Natural plants are gaining acceptance because of their lower toxicity and side effects, as compared to chemical drugs. The Food and Drug Administration (FDA) has approved more than sixty percent of the medications which are derived from natural sources and are used in cancer treatment[6,7]. Numerous classes of extracts and medicinal plant products have demonstrated potential as anti-cancer agents on both *in vitro* and *in vivo* studies[8,9]. The *Rauvolfia serpentina* leaf extract contained many phytochemicals such as stigmasterol, lupeol, campesterol, and phytol resulting in high level of antioxidant with IC_{50} = 146.67 µg/ml by ABTS, IC_{50} = 106.16 µg/ml by DPPH, IC_{50} = 1.4 mg/ml by FRAP. The extract reduced cell viability of human hepatocarcinoma HepG2 and cervical cancer HeLa with IC_{50} = 274.12 µg/ml and 231.33 µg/ml, respectively[10]. *Hibiscus sabdariffa* L. exhibited cytotoxicity effect in Caco-2 colorectal cancer cell with IC_{50} of 17.51 ± 0.07 µg/mL through increased apoptosis[11]. The extract from *Biarum bovei* showed anticancer properties against prostate cancer cells DU-145, human breast adenocarcinoma MCF-7, and HeLa cell lines with IC_{50} ranged between 22.73 and 44.24 µg/ml[12]. In addition, crude *Annona muricata* extract had anticancer activity against breast cancer cell lines by inducing apoptosis and decreased the size of the tumor and weight in a mice model[13].

A medicinal plant, *Shorea roxburghii* is usually found in Thailand, Laos, Cambodia, Myanmar, and Vietnam[14]. Various parts of *S. roxburghii* contain a range of bioactive compounds that offer numerous health benefits. For example, *S. roxburghii* leaves has antidiabetic potential by decreased fasting blood glucose and improved the body weight, food and water intake of treated diabetic rats[15]. The extract also has antioxidant and anti-inflammatory properties demonstrated by decreased renal markers including serum creatinine, blood urea nitrogen, as well as other inflammation molecules in CTX-induced nephrotoxicity rat model[16]. *S. roxburghii* barks have rich sources of antioxidant polyphenolic compounds[17]. Resveratrol-oligomers isolated from them showed potential for anti-cancer through cell cycle arrest and apoptosis induction against SK-MEL-28 melanoma cells[18]. Moreover, two of

twelve compounds isolated from the roots of *S. roxburghii* exhibited anticancer properties against human epidermoid carcinoma Hela and KB cells (IC_{50} values range of 6.5 to 10.1 µg/ml)[19]. However, there have been few recorded investigations into the flower of *S. roxburghii*. The objectives of this study were to analyze the phenolic and flavonoid contents present in methanolic extracts of *S. roxburghii* flowers, as well as to evaluate their antioxidant activity and potential for inhibiting gastrointestinal cancer cell lines.

Materials and methods

Herbal plant collection and identification

In June 2021, flowers of *S. roxburghii* had been collected from local fields in Ubon Ratchathani province, Thailand. The plant was authenticated at the herbarium of Southern Center of Thai Medicinal Plants, located at the Faculty of Pharmaceutical Science, Prince of Songkla University, Songkhla, Thailand. It was assigned the herbarium number SKP 064 19 18 01.

Sample preparation and extraction

Dried *S. roxburghii* flowers were ground into a fine powder and weighed at 450 grams. This powder was macerated in 4.5 liters of methanol for three days. The liquid extract was then filtered through Whatman no. 1 filter paper. Following filtration, the liquid extract was concentrated using a rotary evaporator (Buchi, Switzerland) under vacuum conditions. The resulting methanolic extract of *S. roxburghii* was protected from light and stored at -20°C. Before being used in further experiments, the crude extract was dissolved in 100% DMSO to prepare a stock solution at a concentration of 100 mg/ml.

Total phenolic contents

Adapted from previous research, the Folin-Ciocalteu method was performed to quantify the phenolic contained in the methanolic extract[20]. Specifically, 100 µl of 10% (w/v) Folin-Ciocalteu reagent (Merck KGaA, Darmstadt, Germany) was combined with 20 µl of methanolic extract at a concentration of 1 mg/ml and allowed to incubate for 30 minutes. Following incubation period, 80 µl of 7% Na_2CO_3 was added to the mixture. The absorbance was measured at 750 nm utilizing a microplate reader. Total phenolic content was expressed as micrograms of gallic acid equivalents per milligram of dry extract (µg GAE/mg), with reference to a standard curve using gallic acid (Sigma-Aldrich, St. Louis, MO, USA).

Total flavonoid contents

Adapted from previous research, the quantification of flavonoid content in the methanolic extract was performed utilizing the aluminum chloride ($AlCl_3$) method[20]. A 30 µl aliquot of methanolic extract at concentration 1 mg/ml was combined with 10% (w/v) $AlCl_3$ solution in distilled water at volume of 10 µl, 1M potassium acetate (CH_3CO_2K) at volume of 10 µl, distilled water at volume of 30 µl, and absolute ethanol at volume of 170 µl and incubated for 30 minutes. After incubation at room temperature, the absorbance was measured at 415 nm using a microplate reader. Quercetin, obtained from Sigma-Aldrich (St. Louis, MO, USA), was measured for setting the standard curves. Flavonoid content was expressed as micrograms of quercetin equivalents per milligram of dry extract (µg QE/mg).

Antioxidant assessments by ferric reducing antioxidant power (FRAP) analysis

The antioxidant capacity of the methanolic extract was evaluated using the FRAP assay, following a modified protocol from previous research[20]. The FRAP reagent was freshly prepared by mixing 0.25 M acetate buffer (pH 3.6), 20 mM ferric chloride from Merck (Darmstadt, Germany), and 10 mM 2,4,6-tripyridyltriazine (TPTZ) from Sigma-Aldrich (St. Louis, MO, USA) in a 10:1:1 ratio. The methanolic extract (18 µl) was combined with 182 µl of the FRAP reagent and incubated at 37°C for 30 minutes. The absorbance was measured at 593 nm using a microplate reader. Ascorbic acid, obtained from Solarbio

(Solarbio Science & Technology, Beijing, China), was used for the standard calibration curve. Micrograms of ascorbic acid equivalents per milligram of dry extract (µg AAE/mg) were used to express the antioxidant activity.

Radical scavenging activity by the DPPH (1,1-diphenyl-2-picrylhydrazyl) assay

The antioxidant scavenging activity was assessed using the DPPH assay. The methanolic extract (ranging 250-5,000 µg/ml) in volume of 20 µl was mixed with 180 µl of DPPH reagent (Sigma-Aldrich, St. Louis, MO, USA). After gentle shaking for two minutes, the mixture was incubated at room temperature for 30 minutes. The absorbance was then measured at 517 nm using a microplate reader. Using the following formula, the percentage of radical scavenging activity was determined.

$$\text{Percentage of scavenging effect (\%)} = (A_b - A_s)/A_b \times 100$$

A_b represents the absorbance of the blank reagent, while A_s denotes the absorbance of the extract reaction.

Antiradical curves were produced using the range concentrations of extract. Plotting of these data showed scavenging ability on the y-axis and concentration on the x-axis. EC_{50} values, representing the concentration required to achieve 50% antioxidant activity were calculated and reported using GraphPad Prism version 8.0 for Windows (GraphPad Software, San Diego, CA).

Cell lines and cell culture

This study involved two cancer cell types including gastric cancer and cholangiocarcinoma cell lines. Gastric cancer (GC) cell line, AGS (CRL-1739) was obtained from the American Type Culture Collection (ATCC). For cholangiocarcinoma (CCA) cell line, KKU-100 (JCRB1568) was procured from the Japanese Collection of Research Bioresources (JCRB). The two cell types were cultivated in Ham's F-12 medium, which was supplemented with 10% fetal bovine serum and 100U/ml and 100 µg/ml of penicillin and streptomycin. Cells were maintained in a humidified incubator with 5% CO_2 at 37°C.

Cytotoxicity test

The MTT colorimetric assay was used to perform the cytotoxicity test. A total of 2,000 cells per well were plated in 96-well plates. Following a 24 h incubation, cells were exposed to methanolic extract concentrations ranging from 0 to 200 µg/ml (final DMSO concentration 0.2%) for 48 and 72 h, and then maintained at 37°C in a 5% CO_2 incubator. Following the treatment, cells were washed with PBS and incubated for 2 h at 37°C with MTT reagent. After that, the formazan crystals were dissolved by adding DMSO after the MTT reagent had been withdrawn. The percentage of cell viability was calculated by comparing with the untreated control. The half-maximal inhibitory concentration (IC_{50}), representing the levels of methanolic extract which suppressed the cell until has 50% cell viability, was determined and reported.

Apoptosis assay detected by flow cytometry

Cells were seeded at a density of 100,000 cells per well in 6-well plates. Following a 24 h incubation, the cells were treated with methanolic extract at concentrations of 160 and 320 µg/ml (final DMSO concentration 0.16 and 0.32%, respectively) for 48 h. Control was represented as 0.32% DMSO treated with the same condition. Upon completion of the treatment, the cells were trypsinized and subsequently washed with cold PBS to collect pellet cells. Then, using the Alexa Fluor® 488 annexin V/Dead Cell Apoptosis Kit (Invitrogen™, USA), the apoptotic cell distribution was evaluated. The cells were resuspended in 100 µl of 1× annexin binding buffer, 2.5 µl of Alexa Fluor® 488 annexin V, and 1 µl of 100 µg/ml PI, following the manufacturer's procedure. After staining, flow cytometry was used to evaluate the cells using a FACS Canto II device (BD Biosciences, UK).

Statistical analysis

The quantitative bar chart displaying the half maximal effective concentration (EC_{50}) values and the apoptosis effect, along with the dose-response curve illustrating the half maximal inhibitory concentration (IC_{50}), were calculated and generated using GraphPad Prism version 8.0 for Windows (GraphPad Software, San Diego, CA).

Results

Total phenolic and flavonoid content

The estimation of total phenolic content in the methanolic extract of *S. roxburghii* was conducted using gallic acid as the standard reference compound. The quantification of phenolic content was expressed as micrograms of gallic acid equivalent (GAE) per milligram of extract. The result revealed that each milligram of the methanolic extract contained approximately 161.20 ± 0.66 micrograms of phenolic compounds, as compared to the standard gallic acid. In addition, the determination of flavonoid content was expressed as micrograms of quercetin equivalents per milligram of extract. The analytical data clearly indicated that methanolic extract contain 51.93 ± 5.16 µg QE/ mg dry extract, represent in table 1.

Antioxidant activity assessments

To evaluate the antioxidant activity of the methanolic extract, two well-established assays, the ferric reducing power assay (FRAP) and the DPPH radical-scavenging capacity assay, were performed. In the FRAP, the reducing power of the extract was assessed by measuring its capacity to reduce ferric ions to ferrous ions. The results were expressed as micrograms of ascorbic acid equivalents per milligram of extract, providing a quantitative assessment of the extract's antioxidant capacity. The FRAP values of methanolic extract was 57.31 ± 4.29 µg AAE/ mg dry extract as shown in table 1.

DPPH radical-scavenging capacity assay provides a reliable method for evaluating the ability of extract to quench free radicals. The concentration of antioxidant required for 50% scavenging of DPPH radicals (EC_{50}) was determined for both the methanolic extract and standard ascorbic acid. The EC_{50} value for the methanolic extract was 528.10 µg/ml, which was comparatively higher than that of ascorbic acid as shown in figure 1.

Table 1 Total phenolic, flavonoid content and antioxidant assessed by ferric reducing power method

Shorea roxburghii extract	Total phenolic content (µg GAE/ mg dry wt)	Total flavonoid content (µg QE/ mg dry wt)	Ferric reducing power assay (µg AAE/ mg dry wt)
Methanol	161.20 ± 0.66	51.93 ± 5.16	57.31 ± 4.29

Note: The data are presented by mean ± SD.
Abbreviations: GAE, gallic acid; QE, quercetin; AAE, ascorbic acid; dry wt, dry weight of methanolic extract.

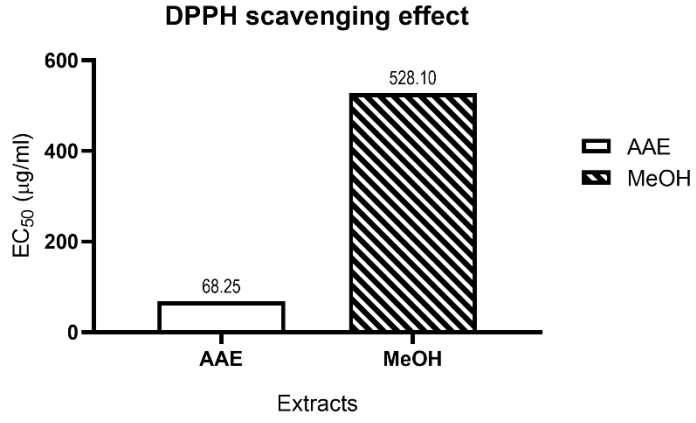

Figure 1 Radical scavenging activity of methanolic *Shorea roxburghii* extract.
EC_{50} values of DPPH scavenging effect in methanolic *S. roxburghii* extract and the standard ascorbic acid.
Abbreviations: MeOH, methanolic extract; AAE, ascorbic acid.

Methanolic extract of S. roxburghii inhibits cell proliferation and induces apoptosis

The cytotoxic effects of the methanolic extract of *S. roxburghii* were investigated on two different cancer cell lines: AGS and KKU-100 representative of GC and CCA, respectively. Following exposure periods of 48 and 72 h, cell viability assessments were conducted utilizing the MTT assay method. Our results demonstrated that AGS cells showed inhibited cancer cell growth with IC_{50} value of 161.6 µg/ml at the 48 h, as shown in figure 2A. The viability of KKU-100 cells was reduced to less than half of the control (untreated with extract) in the presence of 200 µg/ml extract at 48 h, as shown in figure 2B. After 72 h of treatment, AGS cells exhibited the greatest inhibition, displaying an IC_{50} value of 57.81 µg/ml, whereas KKU-100 cells demonstrated an IC_{50} value of 122.5 µg/ml. Our results demonstrated that methanolic extract of *S. roxburghii* had the potential to inhibit gastrointestinal cancer cell growth and gastric cancer was more sensitized compared with CCA. To identify whether methanolic extract inhibited cancer cell growth by inducing apoptosis, AGS cells were selected and treated with crude methanolic extract at concentration 160, and 320 µg/ml. After 48 h of treatment, cells were stained with Annexin V-FITC/PI for flow cytometry analysis. The result showed that the apoptosis rates were (7.0 ± 4.20) %, and (6.9 ± 3.60) %, respectively. On the other hand, the control cells exposed to 0.32% DMSO showed few cells death (0.6 ± 0.05%) in AGS. Our results indicated that methanolic extract of *S. roxburghii* slightly induced AGS cell apoptosis, as shown in figure 3.

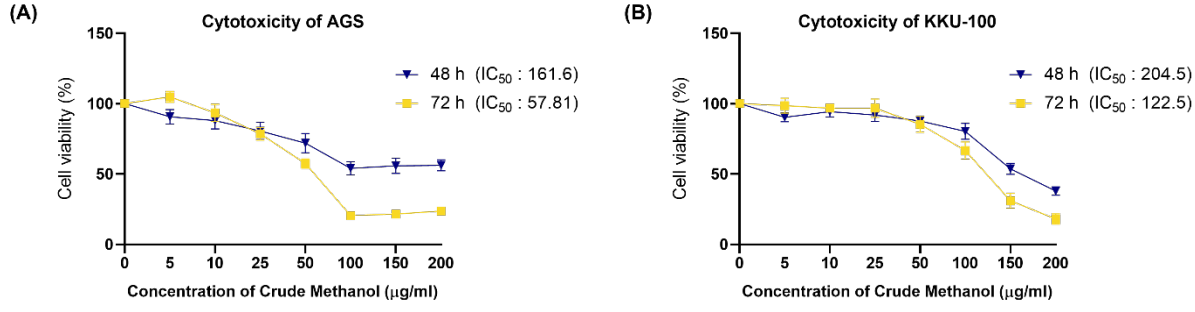

Figure 2 Cytotoxicity of methanolic *Shorea roxburghii* extract with a series concentration 0-200 µg/ml on gastrointestinal cancer cell lines.
(A) AGS treated with methanolic *S. roxburghii* extract in 48 and 72 h
(B) KKU-100 treated with methanolic *S. roxburghii* extract in 48 and 72 h

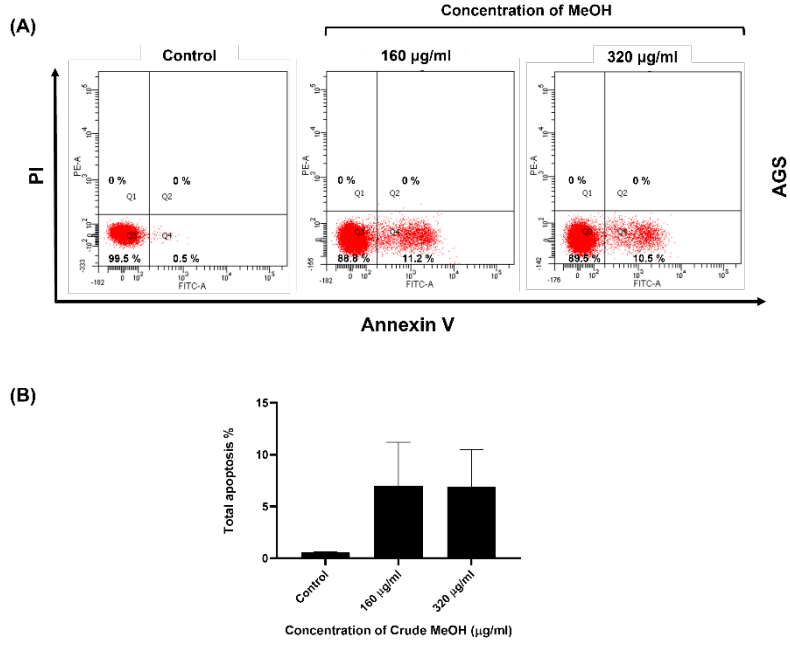

Figure 3 Apoptosis effect of methanolic *Shorea roxburghii* extract.
(A) AGS cells were treated with methanolic *S. roxburghii* extract for 48 h, and apoptosis was determined by flow cytometry using Annexin V/PI double staining
(B) Quantitative analysis of apoptosis is presented by mean ± SEM

Discussion

Plants are organisms that synthesize unique secondary metabolites to survive in various challenging environments. These molecules play crucial roles in the plant's defense mechanisms, as well as provide benefits to other organisms[21]. Phenolics are one of the largest and most diverse groups of secondary metabolites, consisting of compounds with phenol units that play essential roles in plant defense, UV protection, and structural support[22]. Due to their bioactive properties, phenolics are valuable compounds in the

development of functional foods, nutraceuticals, and pharmaceuticals aimed at preventing and treating various diseases. Phenolics are also applied in various defense mechanisms in humans, including as antioxidants, anti-cancer, anti-inflammatory, and cardiovascular protective effects[23].

S. roxburghii is a Southeast Asian plant. Various parts of it contains a variety of bioactive compounds, particularly phenolics, which have garnered attention for its medicinal properties. In our study, we focused on the methanol extract of flowers of *S. roxburghii* to assess their phenolic content and flavonoid contents. The phenolic content in the flower extract was measured at 161.20 ± 0.66 µg GAE/mg, while the stem bark extract reported by Subramanian et al[24] was estimated to contain a total phenolic content of 67.67 ± 4.90 µg/ml, expressed in gallic acid equivalents. Our result indicated that the phenolic content in the methanolic extract of *S. roxburghii* flowers was higher compared to that in the methanol extract of the stem bark. Additionally, flavonoids, one of the phenolic compounds, were found to be 51.93 ± 5.16 µg QE/mg. Supporting our findings, ampelopsin A and H, flavonols belonging to a subclass of flavonoids, were discovered in the methanolic extract of the bark and wood parts of *S. roxburghii*[17]. Other phenolic compounds were also reported in methanolic extract of the *S. roxburghii* such as hopeaphenol, 3-ethyl-4-phenyl-3,4-dihydroisocoumarins, balanocarpol, vaticanols A, and trans-resveratrol[17,25]. This highlights the potential of *S. roxburghii* flowers as a rich source of phenolic compounds that could contribute to their medicinal properties. Phenolic compounds are well-known for their antioxidant properties. They are able to donate hydrogen atoms or electrons to free radicals, thereby neutralizing them and preventing oxidative damage to cells and tissues[26]. Antioxidants play a crucial role in protecting the body against various diseases and aging processes by combating oxidative stress[2]. Our study also investigated antioxidant activity in *S. roxburghii* extract. The result showed that methanolic *S. roxburghii* extract had moderate antioxidant activity with both different methods of detection (FRAP values 57.31 ± 4.29 µg AAE/ mg and EC_{50} value 528.10 µg/ml of DPPH scavenging activity). The result revealed that antioxidant in methanolic *S. roxburghii* extract was higher than that in other medicinal plants such as *Amaranthus viridis* (EC_{50} value 2413.91 µg/ml), *Cordia dichotoma* (EC_{50} value 1145.40 µg/ml), *Sonchus oleraceus* (EC_{50} value 846.75 µg/ml), and *Citrus limon* (EC_{50} value 0.618 mg/ml)[27,28]. This study suggested that the methanolic extract of *S. roxburghii* had substantial antioxidant properties, as evidenced by its ability to reduce ferric ions and scavenge free radicals.

Cancer is a multifactorial disease, with one of the key factors being the accumulation of oxidants. High amounts of oxidants, such as ROS, are known to harm DNA, proteins, and lipids. This damage caused alterations in critical genes, which might lead to the development of cancer cells. Maintaining a balance between oxidants and antioxidants is crucial for preventing oxidative stress and its associated risks[29]. A quarter of all cancer cases worldwide and one-third of cancer-related fatalities are caused by gastrointestinal cancers. Eastern Asians had the highest lifetime risk stomach and liver cancer. This was due to the long-standing high prevalence of certain major risk factors such as smoking, excessive alcohol consumption, *Helicobacter pylori* infection, and liver flukes *Opisthorchis viverrini* infection[30]. Our study investigated the anti-cancer potential of methanolic *S. roxburghii* extract on two different gastrointestinal cancer cell lines. The result demonstrated that methanolic extract of *S. roxburghii* exhibited cytotoxic effects on both AGS and KKU-100 cancer cell lines. AGS cells are more sensitive to the extract compared to KKU-100 with IC_{50} value of 161.6 µg/ml and 204.5 µg/ml, respectively. Based on the observed sensitivity of AGS cells to the methanolic extract

of *S. roxburghii*, AGS cells were selected to investigate the apoptotic effects using flow cytometry. According to Annexin V-FITC/PI double staining, the rates of apoptosis at 160 and 320 µg/ml of crude methanolic extract in AGS were (7.0 ± 4.2) %, and (6.9 ± 3.6) %, respectively. The results suggested that crude methanolic extract of *S. roxburghii* inhibited gastrointestinal cancer cell growth via inducing apoptosis in the cells.

Conclusion

Methanolic extract of *S. roxburghii* flowers is a rich source of phenolic and flavonoid compounds with significant antioxidant and anti-cancer properties. The extract demonstrated moderate antioxidant activity with both FRAP and DPPH scavenging activity. Additionally, the methanolic extract exhibited cytotoxic effects on AGS and KKU-100 gastrointestinal cancer cell lines by induced apoptosis. These findings highlight the potential of *S. roxburghii* as a valuable resource for developing therapeutic agents against oxidative stress and cancer. Its safety, effectiveness, and mechanisms of action in humans, including clinical trial should all be further investigated in more thorough research.

Take home messages

The methanolic extract of *S. roxburghii* showed promising antioxidant and anticancer activities, making it a potential candidate for further research in antioxidant and cancer therapy.

Conflicts of interest

The authors declare no potential conflicts of interest.

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