THE EFFICACY AND SAFETY OF HERBAL MEDICINE COMBINATION FOR MANAGEMENT O F LEG SYMPTOMS DUE TO CHRONIC VENOUS DISEASETHAI PATIENTS: A DOUBLE BLIND ED RANDOMIZED CONTROLLED TRIAL.

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ประสิทธิภาพและความปลอดภัยของตำรับยาสมุนไพรผสมเพื่อรักษาผู้ป่วยไทยที่มีอาการจากสาเหตุโรคหลอดเลือดดำเสื่อมเรื้อรังที่ขา: การศึกษาแบบสุ่มมีกลุ่มควบคุมชนิดผู้ให้และรับการรักษาไม่ทราบชนิดยา

นายอนันต์ อุดมพรประภา

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต
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อนันต์ อุดมพรประภา : ประสิทธิภาพและความปลอดภัยของตำรับยาสมุนไพรผสมเพื่อรักษาผู้ป่วยไทยที่มีอาการจากสาเหตุโรคหลอดเลือดดำเสื่อมเรื้อรังที่ขา: การศึกษาแบบสุ่มมีกลุ่มควบคุมชนิดผู้ให้และรับการรักษาไม่ทราบชนิดยา. (THE EFFICACY AND SAFETY OF HERBAL MEDICINE COMBINATION FOR MANAGEMENT OF LEG SYMPTOMS DUE TO CHRONIC VENOUS DISEASETHAI PATIENTS: A DOUBLE BLINDED RANDO MIZED CONTROLLED TRIAL.) อ.ที่ปรึกษาวิทยานิพนธ์หลัก : ผศ. ดร.เนาวรัตน์ กาญจนาคาร

ตำรับยาสมุนไพรผสม 2% Asiaticoside และ 1% Acemannan ในยาพื้น 2% Acetylsalicylic Acid ในรูปแบบ beeswax encapsulation (HMC) ได้ถูกพัฒนาเป็นยาเจลทาภายนอกเพื่อเป็นทางเลือกในการรักษาโรคหลอดเลือดดำเสื่อมเรื้อรังที่มีความรุนแรงอ่อนและปานกลาง วิธีการศึกษา: ศึกษาเปรียบเทียบประสิทธิภาพละความปลอดภัยของตำรับยา HMC กับตัวยา CONTROL (C) (2% Acetylsalicylic Acid) แบบสุ่มมีกลุ่มควบคุมชนิดผู้ให้และรับการรักษาไม่ทราบชนิดของยา ผู้ป่วย 42 ราย แบ่งสองกลุ่มๆละ, 21 รายจากการรักษาต่อเนื่องนาน 12 สัปดาห์. ประสิทธิภาพวัดโดยค่าคะแนนทางความรุนแรงทางคลีนิกของโรคหลอดเลือดดำ Venous Clinical Severity Score (VCSS), ค่าคะแนนตามอาการที่ประเมินจากความเห็นแพทย์ Physician-Rating Symptoms Perception Score (PRSPS), และผู้ป่วย Patients Self-Rating Symptoms Score (PSSS) รวมถึงคุณภาพชีวิตที่ประเมินโดยแบบสอบถามคุณภาพชีวิตชนิดประเมินผลลัพท์ทางการแพทย์ แบบ 14-ข้อ สำหรับโรคหลอดเลือดดำเสื่อมสมรรถภาพ Chronic Venous Disease (MOS CIVIQ 14), และแบบ 12-ข้อ คุณภาพชีวิตทั่วๆไป (MOS SF 12). ความปลอดภัยระยะสั้น ประเมินจากการแบบรายงานความชุกอาการไม่พึงประสงค์ของผู้ป่วย ตัวชี้วัดปฐมภูมิวัดจากการตอบสนองต่อยาจากการลดลงของค่าแนนความรุนแรงของ VCSS ทางคลีนิกมากกว่าครึ่งหนึ่งนับจากเริ่มได้รับยาสี่สัปดาห์ ตัวชี้วัดทุติยภูมิประกอบด้วยผลการรักษาและอาการที่ดีขึ้นวัดจาก VCSS, PRSPS, PSSS, MOS CIVIQ 14 และ MOS SF12 ตามลำดับ. ผลการศึกษา: ผู้ป่วยไทยที่มีอาการจากโรคหลอดเลือดดำเสื่อมไม่ว่าความรุนแรงทางคลีนิคชนิดอ่อนและปานกลางแบบใด (CEAP1/CEAP2) ไม่ว่าจะมีโรคร่วมที่พบจากการศึกษา หรือลักษณะความรุนแรงของอาการ ตอบสนองต่อตำรับยาสมุนไพรผสมที่ 57.14 % ดีกว่ายาควบคุมที่ 9.52%, การตอบสนองต่อการรักษานี้เกิดจากการได้รับตำรับยาผสมและมีนัยสำคัญทางสถิติ ที่ p=0.003, P=0.003 and p=0.003 ตามลำดับ. ตำรับยาสมุนไพรผสมสามารถลด VCSS ได้ดีกว่า ยาควบคุมอย่างมีนัยสำคัญทางสถิติตลอดสีสัปดาห์ที่ 11.9048(6.4908) VS 22.4702 (7.9438) p<0.001. ตำรับยาสมุนไพรผสมสามารถลด PRSPS และ PSSS ได้ดีกว่ายาควบคุมอย่างมีนัยสำคัญทางสถิติ ที่จากเริ่มรักษาถึงสองสัปดาหี p=0.001 และ p=0.017, ระหว่าง 2-4 สัปดาห์ p=0.001 และ p= 0.004, และระหว่าง 8-12 สัปดาห์ p=0.001 และ p=0.003. ทั้งตำรับยาสมุนไพรผสมและยาควบคุม สามารถทำให้คุณภาพชีวิตที่วัดโดย global and subscore score of MOS SF12 และ MOS CIVIQ-14 ดีขึ้นอย่างมีนัยสำคัญทางสถิติ p<0.001, P<0.001. สำหรับการรายงานความปลอดภัยตั้งแต่เริ่มต้นจนสิ้นสุดการรักษาพบว่าตำรับยาสมุนไพรและยาควบคุมพบไม่แตกต่างกัน(p=0756). สรุปผลการศึกษา: ตำรับยาสมุนไพรผสม 2% Asiaticoside, 1% Acemannan ในยาพื้น 2% Acetylsalicylic Acid ในรูปแบบ Beeswax encapsulation ชนิดยาเจลทาภายนอกให้ผลการตอบสนองทางคลีนิกดีกว่ายากลุ่มควบคุม ตำรับยาสมุนไพรผสมและยากลุ่มควบคุมมีความปลอดภัยไม่แตกต่างกันกัน

ภาควิชา	ไม่สังกัดภาควิชา/เทียบเท่า	ลายมือชื่อนิสิต .....
สาขาวิชา	วิทยาศาสตร์สาธารณสุข	ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก .....
ปีการศึกษา	2561	

5679051053 : DOCTOR OF PHILOSOPHY
RANDOMIZED CONTROLLED TRIAL, HERBAL MEDICINE COMBINATION, CHRONIC VENOUS DISEASE, LEG SYMPTOMS, ASIATICOSIDE, ACEMANNAN, ACETYLSALICYLIC ACID, TOPICAL GEL PREPARATION, HEALTH-RELATED QUALITY OF LIFE OUTCOMES

Anan Udombhornprabha :

THE EFFICACY AND SAFETY OF HERBAL MEDICINE COMBINATION FOR MANAGEMENT OF LEG SYMPTOMS DUE TO CHRONIC VENOUS DISEASETHAI PATIENTS: A DOUBLE BLINDED RANDOMIZED CONTROLLED TRIAL.. ADVISOR: Asst. Prof. Naowarat Kanchanakhan, Ph.D.

The herbal medicine combination containing 2% Asiaticoside and 1% Acemannan in a 2% Acetyl Salicylic Acid in beeswax encapsulation as an ACTIVE treatment (HMC) was developed as a topical gel preparation as treatment options for Chronic Venous Disease (CVD) with mild-to-moderate severity. Methods: The efficacy and safety of HMC as compared with a CONTROL treatment (C) (2% Acetylsalicylic Acid) was investigated in a double-blind randomized controlled trial, 42 CVD patients, 21 of whom allocated either HMC or C, for 12-week continuous treatment. The efficacy assessment employed Venous Clinical Severity Score (VCSS), Physician-Rating Symptoms Perception Score (PRSPS), Patients Self-Rating Symptoms Score (PSSS) and two types of short-form health-related quality of life scores, a Medical Assessment Study, 14-item Chronic Venous Disease (MOS CIVIQ 14), and a 12-items Genearal Health (MOS SF 12). The short-term safety assessed with patients-self reported adverse events. The primary endpoint was a responder rate defined by 50% reduction of the VCSS after 4-weeks of treatment from baseline. The secondary endpoints assessed improvement of overall sympotms assessed by VCSS, PRSPS, PSSS, MOS CIVIQ 14 and MOS SF12 accordingly. Results: CVD Thai patients(N= 42) in general and regardless of clinical severity class (CEAP1/CEAP2) or Co-morbidity responded 57.14 % to HMC(N=21) as compared with 9.52% for C(N=21), the responder rate was significantly dependent on intervention, p= 0.003, P= 0.003 and p= 0.003 respectively. HMC reduced VCSS significantly better than C, overtime at 4-weeks 11.9048(6.4908) VS 22.4702 (7.9438) p<0.001. HMC reduced PRSPS and PSSS significantly better than C, at Basline to 2-weeks p=0.001 and p=0.017, 2-4 weeks p=0.001 and p= 0.004, and 8-12 weeks p=0.001 and p=0.003 respectively. After 12-weeks from baseline, both global and subscore for MOS SF 12 MOS CIVIQ-14, treatment with HMC and C significantly improved at p<0.001, p<0.001 (MOS CIVIQ 14) respectively. For adverse drug events reported, no statistical significant different between HMC and C from baseline to the end of 12-week follow-up (p= 0.756). Conclusion. The herbal medicine combination containing 2% Asiaticoside, 1% Acemannan in 2% Acetyl Salicylic Acid in Beeswax encapsulation (HMC) as a gel preparation topical administration provided clinical responder rate better than the Control. Both HMC and C were not significant different in terms of safety assessment.

Department:	Common Course	Student's Signature .....
Field of Study:	Public Health Sciences	Advisor's Signature .....
Academic Year:	2018	

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This thesis investigation entitled “THE EFFICACY AND SAFETY OF HERBAL MEDICINE COMBINATION FOR MANAGEMENT OF LEG SYMPTOMS DUE TO CHRONIC VENOUS DISEASE THAI PATIENTS: A DOUBLE BLINDED RANDOMIZED CONTROLLED TRIAL” owed categorical supports from the Chulalongkorn University Research Funds and the College of Public Health Sciences, Chulalongkorn University to whom my thesis advisor Assistant Professor Dr. Noawarat Kanchanakhan Ph.D., and Professor Surasak Taneepanichsakul M.D., were the two key supports behind this successive work. I also owed tremendous supports from the research investigation team members, a Surgean Dr. Pichai Phongmanjit M.D., two researcher nurses Mrs. Onnapa Yodkharn B.N.S. and Mrs. Peerapich Kaewlung B.N.S. and several others administrative members from Chiang Rai Prachanukhroh Hospital, the Somdej Prayannasangworn Hospital SPDY, Chiang Rai whom tirelessly commited with trusts from the initiation that this investigation successfully completed with uncompromised for the difficulty in recruiting the right patients, and their tremendous works in conducting all steps required as per the rigid research protocols especially an impeccable works in data completion. This innovative works could not succeed without an early works for laboratories and test product manufacturing supports from the Labs of the School of Pharmacy Eastern Asia University, the Dean, Associate Professor Papavadee & Asst. Professor Vichien Thanindratarand, BSPharm, MPharm (Pharmaceutics) and Associate Professor Dr. Nongluks Sriubolmas BSPharm, MSc (Microbiology), PhD (Microbiology).

The last but not least, I would like to thank all patients whom instilling our best knowledge in realworld clinical setting for this research works including how medication adherence came into play, as patient reverberated “Its good that’s why I need it more”. Our endeavor to foster patients to take medication what they really need and to stop what they don’t need was the most challengeing tasks for real world patient medication counseling.

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INTRODUCTION

1.1 BACKGROUND

Chronic venous disease (CVD) is a global health burden especially in the West which reported the presence of varicose veins as high as 20-33%. In many cases, clinical history and physical examinations looking for patient's symptoms severity could help confirm diagnosis of CVD [1,2]. In Thailand, a high incidence of superficial varicose vein of 32.99% or so had been reported, however these patients were not usually presented with serious problematic leg symptoms. In addition, many of most probable CVD diagnosed patients did not seek medical treatments as had been reported among female factory workers particularly in Thailand [3]. Moreover, the important findings had been reported that the hospitalized leg ulcer patients in Thailand were mainly diagnosed with late stage CVD with leg ulcers, primarily of venous origin which often due basically to the untreated CVD [4]. Nevertheless, these problematic leg symptoms reported by specialists in Thailand were still largely varied differently among both young and older CVD patients [5]. An epidemiological study at primary care setting for probable patients diagnosed with chronic venous insufficiency (CVI), had reported problematic leg symptoms such as heaviness in the legs, itching as their common symptoms [6]. Though gross appearances of cutaneous skin lesions such as varicose veins were essential to confirm the diagnosis of CVD,

other supports such as concurrent presence of troubling symptoms should also be helpful to secure CVD diagnosis [7]. In practice, many patients with venous insufficiency did not receive proper early diagnosis and thereby early treatment [8]. Inadvertently, many of such silent CVD patients was remain under-diagnosis and under-treated in some way, whereby patients still seek symptomatic treatment with other systematic drugs especially various types of pain-killers. The above was dued basically to unavailable of effective standard medication for treatments of CVD. The current treatment modalities were primarily employing compression stocking and encouraging patients' lifestyle self-modifications. It was so clearly observed, particularly if such CVD patients' condition was not severe enough to deserve expensive surgical procedure. Several studies involving CVD and or CVI have been concurrently reporting poor health-related quality of life (HRQOL) among individual diagnosed with CVD and or CVI. These were widely investigated by many authors elsewhere [9-14]. As results, these findings have been strongly suggesting the needs for early diagnosis and early treatment of CVD as preventive issue. Subsequently, the former would be allowing early spotted CVD and the later the effective treatment of CVD with the implication of improving HRQOL and preventing later ongoing of the disease process. Since there has been no effective standard medication for management of CVD, an attempt to develop medication especially the herbal-base medicine, that should be safe and effective to alleviate overall problematic leg symptoms of CVD had been initiated. Subsequently, the literature

reviews for possible selection of probable safe and effective herbal-based medicinal agents were performed through electronic database searching via PubMed, Medline, Google Scholar and the Cochrane Library. The search query began with “drug treatment of chronic venous disease” filtered with subjected to clinical trial, available as at least abstracts and investigation only in human since 1960 to 2015. There were only 356 articles founded. Subsequently, an exhaustive screened through each of the abstracts to exclude the overlapping CVI reported within either the domain of other cardiovascular disease or CVI mimic with venous leg ulcers. Subject to data exclusion, with consultations and agreements among researchers for the search data with a vascular specialist were conducted. It was noteworthy that, despite many of such studies with connection to efficacy and safety of herbal-based agents had already been reported, there were still reflection of conflicting evidences of whether there was the true efficacy of herbal drugs as due mainly to inconclusive or inexplicable mechanism of actions or the other. The above was probably due to limitations of study designs. Among these active herbal medicinal agents, two major active herbal extracts namely (1) Titrated Extract Centella Asiatica (TECA) – Asiaticoside (2) Acemannan (Aloe vera) were identified as the most likely safe and effective and a truly herbal extract. In addition, possibility of bringing Acetylsalicylic Acid (ASA) pharmaceutical base with enhance skin absorption and possibly render therapeutics effects through microencapsulation was developed. These selections were based on possible synergistic of different mechanism of actions combined as

intended for anti-inflammation, anti-fibrosis and anti-coagulation, antibacterial and immunomodulation [15-25,111-122.142-150]. These two herbal-medicine extracts were obtained through US - FDA verified plants extracts globally commercial source, to ensure the extracts contents to allow precision in formulation. The herbal medicine combinations topical formulation was formulated later as a pilot-scale by incorporation of the two herbal extracts in combination into the ASA microencapsulation bases to become a finished product for clinical investigation in CVD patients. In a later stage, a research protocol for hospital ethical approval to get patient accessibility was submitted and being granted to investigate at Somdet Phra Yanna Sangworn (SDPY) Hospital Wiang Chai, and Chiang Rai, Hospital Thailand. The investigation clinical trial was thereby registered within biomed central domain as ISRCTN No.54360155 [28].

1.2 RATIONALE

The rationales to conduct clinical investigation of 2% Titrated Extract of Centella Asiatica **(**TECA**)** and 1% Acemannan within a 2**%** ASA microencapsulation bases by looking at efficacy and safety in reduction of the leg symptoms due to CVD in Thai patients suffering are as following:

1. The first and foremost, since there is neither effective standard medical treatment particularly topical formulation had ever been developed for

treatment of CVD. Thus, this is the first protocol clinically developed with herbal-medicine formulation for topical management of leg symptoms of CVD.

2. Though numbers of herbal-medicine and herbal extracts intended for systematic administration as medical treatments for CVD are available, the outcomes of such clinical trials provided inconclusive evidence. In addition, several herbal-based products were released into market despite some anecdotal reports with respect to efficacy and safety. Moreover, the applications of both compression stocking either elastic or non-elastic bandage which remains the standard options despite its inconvenient administrations had been encountered by patients and regarded as noncompliance in the long-term. Likewise, clinical limitations of various treatment modalities were limited due to current cost containment. The lack of awareness campaign for CVD diagnosis and its recognition in conjunction with current high cost of intervention not withstanding complicated surgical procedures if any, should have been emerging to bring about future healthcare cost burden of the Thai healthcare system. There are essentially myriads of benefits and should therefore need for dual approach as for (a) an early awareness in terms of diagnosis and (b) an early treatment of CVD [29,30].
3. Aloe vera, with Acemananan as active polysaccharide has been regarded as an economic herbal plant in Thailand. Aloe vera and Acemannan are safe and well-known traditional herbal medicine. Aloe vera drinks are well-known Thai

traditional herbal drinks. This investigation could enrich the clinical profiles of Aloe vera, thereby should be valuable for existing therapeutic potential for further development in many diseases including CVD [15-22].

4. Centella Asiatica, containing Titrated Extracts of Centella Asiatica (TECA) has been regarded as one of a traditional vegetable food compliment in Thai food. In Thailand, traditional water extract of Centella Asiatica is a common herbal drink. In Europe, TECA extracts had been developed as cosmetics product for the external application as treatment of varicose veins whereas the systemic administration of the same for CVD has some limitations in clinical trial setting in clinical efficacy study due to safety despite its potential anti-thrombotic properties [23-25].
5. Acetylsalicylic Acid (ASA) – pharmaceutical base, is well recognized for preparation to support medicinal for enhance skin absorption. ASA originally known as pure extracts from Willow Bark with its putative mechanism of action as anti-inflammatory and anti-platelet orally, and probable positive efficacy in leg ulcers, however shortcomings of ASA via systematic administration remained to be solved. Whether, ASA could accelerate ulcer healing among venous ulcers have not been investigated in mild to moderate CVD which demands further clinical investigations [26,27]. The availability of innovative formulation of ASA-base in microencapsulation for topical preparation would allow the other two herbal extracts combination possible innovative development.

6. Primarily, the ASA-base topical formulation in microencapsulation procedure had been developed. Then the combination with other two stable herbal-medicine extracts had been incorporated. The rationale of this study is to assess the efficacy and safety of the herbal-medicine combination topical preparation which contains (1) 2% Titrated Extract Centella Asiatica (TECA)-Asiaticoside (2) 1% Acemannan (Aloe vera) in a topical 2% ASA microencapsulation gel bases for management of leg symptoms due to chronic venous disease Thai patients. This study should provide supporting evidence if the herbal-medicine combination adding two herbal medicine extracts to ASA-microencapsulation base as above should be further developed as medical treatment for effective relief of leg symptoms due to CVD.

1.3 RESEARCH GAP

There is no herbal-medicine topical preparation as therapeutic alternative for treatment of problematic leg symptoms caused by CVD. Although, available herbal plants, as may possibly inherited therapeutic potential for CVD has been reported, there is no conclusive evidence and thus demands further research and development. The topical formulation of herbal-medicine combination containing 2% Titrated Extract of Centella Asiatica (TECA), 1% Acemannan added to a 2% Acetylsalicylic Acid (ASA) bases could be formulated and prepared as an extemporaneous preparation and for further clinical investigation.

1.4 RESEARCH QUESTION

"Is the herbal-medicine topical preparation containing 2% Titrated Extract of Centella Asiatica (TECA) and 1% Acemannan added to a 2% ASA bases effective in reduction of the problematic leg symptoms due to chronic venous disease (CVD) better than 2% ASA microencapsulation bases alone as a control preparation?"

1.5 RESEARCH OBJECTIVE

Primary Objective:

To assess the overall responder rate as effects of the topical application of the herbal medicine combination containing 2% Titrated Extract of Centella Asiatica (TECA) and 1% Acemannan in a 2% ASA bases in reduction of the leg symptoms due to Chronic Venous Disease.

Secondary Objectives:

1. To assess the responder rate as effects of the topical application of the herbal medicine combination containing 2% Titrated Extract of Centella Asiatica (TECA) and 1% Acemannan in a 2% ASA bases in reduction of the leg symptoms due to CVD based on patients CEAP class as severity of Chornic Venous Disease.

2. To assess the responder rate as effects of the topical application of the herbal medicine combination containing 2% Titrated Extract of Centella Asiatica (TECA) and 1% Acemannan in a 2% ASA bases in reduction of the leg symptoms due to Chronic Venous Disease based on patients' background presence of co-morbidity.
3. To assess the responder rate as effects of the topical application of the herbal medicine combination containing 2% Titrated Extract of Centella Asiatica (TECA) and 1% Acemannan in a 2% ASA bases in reduction of the leg symptoms due to Chronic Venous Disease based on patients' background presence of co-morbidity and CEAP severity combined.
4. To assess the overall effects of the topical application of the herbal medicine combination containing 2% Titrated Extract of Centella Asiatica (TECA) and 1% Acemannan in a 2% ASA bases in reduction of the leg symptoms due to Chronic Venous Disease based on (4.1) Venous Clinical Severity Score reduction (VCSS), (4.2) Physician-Rated Symptoms Perception Score (PRSPS) reduction (4.3) Patients Self-rated Symptoms Score (PSSS) reduction and (4.4) Health-related Quality Of Life score assessed with (4.4.1) a General health score, a Medical Outcomes Study 12-item Short Form Questionnaires (MOS SF-12) (4.4.2) a Disease specific score, a Medical Outcomes Study 14-item Short Form Questionnaires for Chronic Venous Disease (MOS CIVIQ-14).

5. To assess the overall patients'self-reported of presence of adverse effects as presence or absence.

1.6 RESEARCH HYPOTHESIS

"The response to treatment of mild-to-moderate Chronic Venous Disease (CVD) with a herbal medicine combination topical application containing 2% Titrated Extract of Centella Asiatica (TECA) and 1% Acemannan with in a 2% ASA bases provided 35% better response as compared with a 2% ASA bases as a control."

1.7 RESEARCH DESIGN

The research design was an interventional, prospective randomized, double-blind, controlled trial. Patients were prospectively recruited and the interventions were concealed allocation either with active or controlled, as double-blind between patients, nurse and surgeon by employing computer-generated set of two randomized numbers through consecutive randomization.

1.7.1 Intervention

Participants fulfilling the eligibility criteria were randomly allocated into one of two arms.

1.7.1.1 Active treatment arm: each of participants were given herbal medicine combination gel formulation containing 2% Asiaticoside and 1% Acemannan in a 2% ASA base. It was applied to the leg with symptoms once in the morning and then again at bedtime continuously for 3 months or 12 weeks.

1.7.1.2 Control treatment arm: each of participants were given a 2% ASA base as controlled-gel with identical odor and color with no active herbal drugs (without 2% Asiaticoside and 1% Acemannan). It was applied to the leg with symptoms once in the morning and again at bedtime continuously for 3 months or 12 weeks.

1.7.2 Health outcome measures [32-38]

1.7.2.1 Primary outcome measures [32,33]

Responder rate (%) looking at venous disease severity, assessed by the Venous Clinical Severity Score (VCSS) measured at week 4 with 50% reduction after baseline score [32,33].

1.7.2.2 Secondary outcome measures [32-38]

Disability caused by venous disease, assessed by health-related outcomes as follows:

(**1**) the Venous Severity Clinical Score responder based on CEAP severity, presences of patients' comorbidity and both disease and venous clinical severities assessed by VCSS at Week 4,8 and after the end treatment (Week 12)

(**2**) the Physician-Rated Symptom Perception Score (**PRSPS**) measured at baseline, week 1,2,3,4,8 and 12 and [34,35].

(**3**) Patients-Self-Rated Symptom Scale (**PSSS**), measured at baseline, week 1,2,3,4,8 and 12 [34,35].. The former (1) was an objective assessment graded by surgeon

whereas the latter (2 and 3) were an adapted disease specific symptoms and a visual analog scale (VAS) for each symptom. Each of categories of VAS measured in length (as centimeter) and the final total score combined employed a transformed percentage scores from absent (0) to severe (100) as subjective self-assessment of selected relevant symptoms rated by patients [34,35].

(**4**) The Health-Related Quality of Life (HRQOL), was a medical outcomes study (MOS) assessed by a Thai validated version of both (4.1)a self-rated 14-Item Short-form Health Survey Questionnaires developed by Chronic Venous Insufficiency Quality of Life Scale International Collaboration with 14 questionnaires (MOS CIVIQ-14). The MOS CIVIQ 14 score shall be presented for comparison [36,37] (**4.2**) a self-rated 12-Item Short-form Medical Outcomes Study., (MOS SF-12) [38]. These two HRQOLs were assessed at baseline and after the last visit at week 12.

(**5**)The summary of adverse events symptoms reported as absence/presence in weekly diary report.

1.8 CONCEPTUAL FRAMEWORK

1. Patients diagnosed as CVD as per CEAP criteria by a medical specialist, hospital practitioner surgeon in a routine OPD clinics. 2. CVD patients met with eligibility criteria fulfilled with inclusion-exclusion criteria, then given detailed intention to participation performed by two researcher nurses, each participant was willing to sign and provide patient-informed consent. 3. Patients were given

details for each treatment procedure before randomization and further appointment were attempted for follow-up accordingly. 4. Treatment allocation was given in an envelope for each patient which included randomized number. 5. Randomization was blinding for investigators (surgeon, nurses and patients) where products blinding concealment code randomized by a set of two and a series of product in each of the following follow-up weeks in a consecutive randomization.

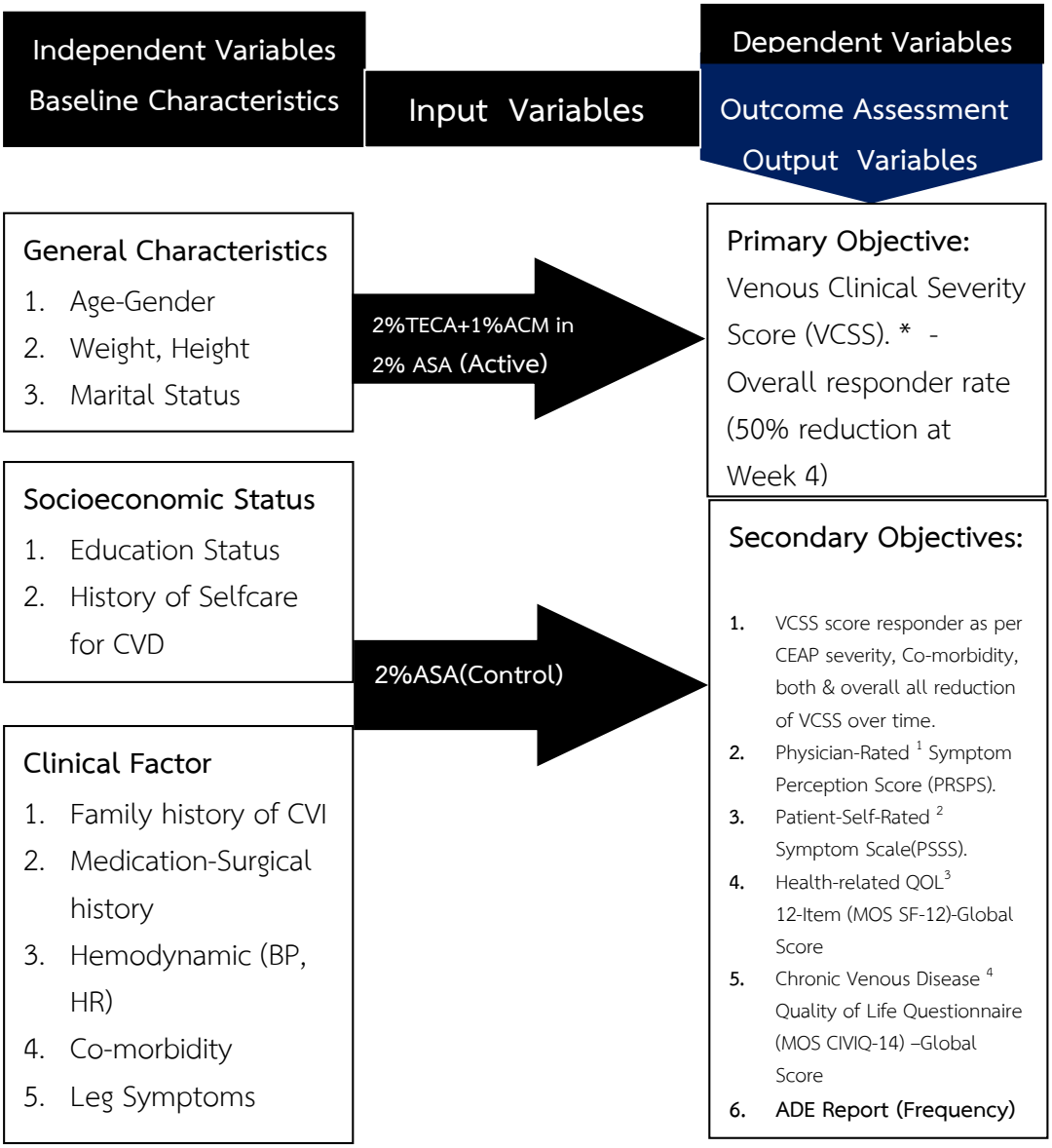


Diagram 1 *Conceptual Framework*

Note. **CEAP = Comprehensive Classification System for Chronic Venous Disorders which CEAP classification include a description of the clinical class (C) based upon objective signs, the etiology (E), the anatomical (A) distribution of reflux and obstruction in the superficial, deep and perforating veins, and the underlying pathophysiology (P), whether due to reflux or obstruction [31]. *Duplex Diagnostics employed for differentiation in selected cases [1, 2, 3, 4] Validated Thai Version; # Mean weekly ADR reported by patients**

1.9 OPERATIONAL DEFINITION

1. Chronic venous disease patients are prospective patients recruited and diagnosed by surgeon as per CEAP criteria subjected to eligibility requirement and fulfilled inclusion and exclusion fulfillment [31].

2. Outcome assessments (OS) comprise of following (details given in research tools):

2.1 VCSS, the **Venous Clinical Severity Score** as suggested by Rutherford et al [32] and later confirmed for its specificity by Meissner et al [33] were objective assessment questionnaires for specialist to evaluate the seven domains of chronic venous insufficiency symptoms and characteristics. These symptoms included (1) pain, (2) varicose veins, (3) venous edema, (4) skin pigmentation, (5) inflammation (6) induration (7) presence of active ulcers including numbers of ulcers and duration of ulcerations. The score ranges from 0 (absence) to severe score (3). Each of the item of venous clinical severity score must be converted into percentage score representing overall severity from 0 to 100, as such the final score computation employed a transformed percentage scores from absent (0) to severe (100). In each items of the score were transformed into percentage score by multiplication of 25 percentage points to each of raw score eg. Raw score 0, then percentage score was 25X0 = 0 whereas then Raw score =1, 2 or 3 then percentage score was 25X1 = 25

points, 25X2 =50 points and 25X3=75 points respectively. Overall VCSS was the summation of the overall clinical severity domain as percentage score [32].

2.2 PRSPS, the **Physician-Rated Symptom Perception Score** as suggested by Talley et al [34] was adapted to CVD which was an objective assessment questionnaire of disease specific symptoms for specialist to evaluate the selected all of any relevance clinical symptoms included (1) Heavy leg (2) Pain in the leg (3) Sensation of leg itching (4) Night cramp (5) Itching leg (6) Sensation of burning (7) Sensation of Needle in the leg (8) Superficial varicose veins (9) Presence of spider veins. The PRSPS was a modified adapted with disease specific symptoms adjustment for chronic venous disease. The score ranged from 1(none) to most severe score (5). The PRSPS score assessed as symptoms severity as such the final score computation employed a transformed percentage score ranging from absent (0) to severe (100). In each items of the score were transformed into percentage score by multiplication of 20 percentage points to each of raw score e.g.. Raw score 1, then percentage score was 20X1 = 20 whereas then Raw score =2,3,4 or 5 then percentage score was 20X2 = 40 points, 20X3 =60 points, 20X4=80 points and 20X5 = 100 points respectively. Overall PRSPS score was the summation of the overall symptoms severity score for all reported symptoms as percentage score [34,35].

2.3 PSSS, the **Patients-Self-Rated Symptom Scale** was a visual analog scale from 1(none) to 10(the most severe), it was a subjective self-assessment of all selected most relevant patients perceived CVD symptoms included (1) Pain (2) Heavy leg (3) Itching in the leg (4) Needle or Pin in the leg (5) Night cramp (6) Leg edema and (7) Any other reported symptoms. These were measured in centimeters rated by patients as suggested by Talley et al [35] with a modified selected CVD symptoms. The final score computation employed a percentage scores from 0 to 100 for interpretation. The PSSS score assessed as symptoms severity as such the final score computation employed a transformed percentage score ranging from absent (0) to severe (100). In each items of the score were transformed into percentage score by multiplication of 10 percentage points to each of raw scales e.g.. Raw scales 1, then percentage score was 10X1 = 10 whereas then Raw score =2,3,4 and to 10 then percentage score was 10X2 = 20 points, 10X3 =30 points, 10X4=40 points and to 10X10 = 100 points respectively. Overall PSSS score was the summation of the overall symptoms severity score for all presented symptoms as percentage score [34,35]

2.4 MOS SF-12, was a self-rated health survey by patients for objective assessment of health-related quality of life, known as the 12-item Short Form Medical Outcomes Study or in short known as **SF-12** proposed by Ware JE et al [38]. In this investigation, The MOS SF-12 Thai version, as a validated

translation was employed. The MOS SF-12 scoring was employed for MOS SF-12 similar to SF 12 for physical function (PF). role limitations because of physical health (role-physical, RP), bodily pain (BP), general health perceptions (GH), vitality (VT), social functioning (SF), role limitations due to emotional problems (role-emotional, RE), and mental health (MH). The scoring domains divided into Physical Component Summary Score (PCS) (Items 1,2,3,4,5 and 8), Mental Component Summary Score (MCS) (items 6,7,9,10,11 and 12) and global score (all items). The computation of score were computed as assigned percentage scoring from each item response yes/no (1/0) and scoring from a categorical response choice (1 to 3, or 1 to 5) and then converted to assigned percentage score between 0 to 100 on each items and each domain, higher scores reflected a better health-related quality of life. The MOS SF-12 percentage score were employed for comparison between the active and control treatment as well as comparison effects of treatment from baseline (day 0) to the end of trial (week 12).

2.5 MOS CIVIQ-14, was structured specific health survey questionnaires for chronic venous disease patients by patients for assessment of health-related quality of life with 5-point Likert scale to rate the importance of leg problem, originally was a 20-items and had been further re-assessed with original adaptation to a 14-item which measured physical, psychological and social functioning and pain known as the CIVIQ-14, an international disease specific health-related

quality of life score [36,37]. There were 14 items which divided into (1) Pain Score (item1), (2) Physical Function Score (items 2,3,4,5,6,7 and 8), (3) Psychological and Social Function Score (items 9,10,11,12,13 and 14). There are 14 questions in the CIVIQ-14, each with 5 possible answers (1 to 5), the minimum possible score being 14 and the maximum 70. In order to calculate the Global Index Score (GIS), the difference between the final score and the minimum possible score was divided by the difference between the theoretical maximum and minimum scores (70-14=56), multiplied by 100. GIS = ([Final score – minimal possible score] / [Theoretical maximal – minimal score]) x 100. The final responding physical function, psychological and social function score were assigned with a percentage point corresponding with each items used in the scoring similar to global index scoring as suggested by authors. For, this investigation, the computation of MOS CIVIQ 14 were reflected as Pain Score, Physical Function Score., Psychological and Social Function Score and Global MOS CIVIQ-14 score. The score comparison reflected both the active and control treatment arms from baseline (day 0) and at the end of the trial (week 12) as well as treatment from baseline to week 12. This investigation employed the Thai version of validated translation CIVIQ 14.

2.6 ADE was the adverse drug event pre-specified self-reported by patients as a weekly summary of follow-up.

2.7 Responder rate is a ratio of number of patients responded to treatment (to be defined by 50% reduction of Venous Clinical Severity Score, or VCSS in 2.1 as compared with baseline score after 4 weeks of treatment) against all patients treated by the same.

2.8 The preparation of herbal medicine combinations topical formulation. The herbal medicine combination was formulated employing the industrial standard herbal extracts (TECA, Asiaticoside (*Centella asiatica* (L.) Urban., Asiatic Pennywort, Gotu Kola, Pegaga, and Acemannan from Aloe vera *barbadensis* Mill., *Aloe indica* Royle, *Aloe perfoliata* L. var. *vera* and *A. vulgaris* Lam)). Both herbal extracts were purchased from US-FDA verified international commercial sources, whereas pharmaceutical formulation of an ASA employed verified pharmaceutical grade as synthesis (NOVACYL®), from which the microencapsulation stabilization process employed the Dimethyl Isosorbide Dinitrate (DMN) as 3% in the overall formulations, thanks to its properties as potent anti-oxidant especially suitable in formulation of ASA in liquid formulation and thus its safety in skincare products [39-43]. The finished preparation was tested and passed for physical stability and microbial safety test which performed in the same laboratory. The aseptic packaging of 35-40 mg herbal-medicine combination gel preparation each in a 45-mg tube, also with labeling for further investigation. The same for controlled products where no herbal active ingredients besides a 2% ASA microencapsulation base.

1.10 EXPECTED BENEFITS AND APPLICATION

Should, the investigation, topical application of the herbal-medicine combination containing 2% Titrated Extract of Centella Asiatica (TECA), 1% Acemannan in a 2% ASA microencapsulation base in reduction of the leg symptoms due to chronic venous disease provide better response rate than that of 2% ASA bases as a Control alone, further development should be pursued. This investigation could foster further research in terms of supporting the development of innovative herbal-medicine as another safe and effective alternative for treatment of chronic venous disease. This herbal-medicine combination formulation is expected to foster national promotion as value-added for herbal plants extract previously known as conventional food and drinks. And thus, may be employed as therapeutics alternative treatment for leg symptoms suffering from mild to moderate chronic venous disease.

CHAPTER II

LITERATURE REVIEW

2.1 CHRONIC VENOUS DISEASE

Chronic venous disease (CVD) of the lower extremities is a common under-diagnosis health problem due basically to its gradual impacts and minimal level health burden in the early stage. The manifestations of CVD range from various physical signs of skin on the legs and presences of various apparent symptoms. The on-going pathological processes of CVD could have severe implications if untreated especially due to inadequate recognition of their various early manifestations from both primary and secondary venous disorders.

The socioeconomic impacts of CVD mainly are due to works-loss disability and productivity losses. In addition, there should have particularly high economic impacts since the CVD is highly related to the large numbers of afflicted factory workers. The untreated as well as inadequately treated CVD could lead to impairment of venous returns earlier and thus bring about chronic venous insufficiency (CVI). CVI is a major cause of venous leg ulcer in the long- term. In the west, chronic venous disease has high prevalence with physical presenting of varicose veins which could have been spotted in an early screening for disease awareness. There are numerous researches and developments despite limitations and lack of innovative medical treatment for CVD. In Thailand, apparent high incidence of superficial varicose vein of 32.99% had been reported among female

factory workers. In spite of this, these workers may not experience troublesome leg symptoms and thereby do not seek early treatment as should for preventing disease progression [3, 43-47].

2.1.1 Definitions and general classifications

CVD can be defined generally as disease of the lower limbs with presenting pathological signs of the skin and concurrent leg symptoms. Their symptoms and signs manifestations range from leg edema, venous eczema and some degree of hyperpigmentation of the ankle, white scar tissue of skin and lipo-dermato-sclerosis or inflammation due to fibrosis of the subcutaneous fat underneath the skin. The most common notable physical signs are the presence of varicose veins and venous ulcers but not always the case. This was basically needed for further differential diagnosis whether it was either the primary or secondary type varicose vein. CVD can be classified or graded as per the descriptive **C**linical, **E**tiologic, **A**natomical, and **P**athophysiological**(CEAP)** classification, which provides some clinical framework for scientific communication to allow appropriate management decision making [31]. The clinical signs in the affected legs are categorized into seven classes designated from graded or classed C0 to C6. The leg symptoms associated with chronic venous disease include aching, heaviness, a sensation of swelling, and skin irritation, limbs categorized in any clinical class may be symptomatic (S) or asymptomatic (A). CVD encompasses the full spectrum of signs and symptoms associated with classes C0 to C6, whereas the term "**chronic venous insufficiency –**

CVI" is generally restricted to disease of greater severity (**classes C4 to C6**). CVI has always been reflected some level of persistent venous hypertension causing various pathologies, including pain, edema, skin changes including advance signs and symptoms of CVI such as hyperpigmentation, venous eczema, lipo-dermato-sclerosis, atrophie blanche, and healed or active ulcers.

2.1.2 CEAP Classification [31]

The CEAP Classification guideline given by the consensus agreement of the revision of CEAP Classification are provided in the table overleaf.

Clinical Classification	Etiologic Classification	Anatomic Classification	Pathological Classification
C0: no visible or palpable signs of venous disease	**Ec:** congenital	As: superficial veins	**Basic CEAP** Pr: reflux
C1: telangiectasies or reticular veins	**Ep:** primary	Ap: perforator veins	**Basic CEAP** Po: obstruction
C2: varicose veins	**Es:** secondary (postthrombotic)	Ad: deep veins	**Basic CEAP** Pr, o: reflux/obstruction
C3: edema	**En:** no venous cause identified	An: no venous location identified	**Basic CEAP** Pn: no identifiable venous pathophysiology
C4a: pigmentation or eczema C4b: lipodermatosclerosis or atrophie blanche			**Advanced CEAP: + any of 18 venous segments /pathology** **Superficial veins Telangiectasies or reticular veins** :1. Great saphenous, 2.Small saphenous, 3. Nonsaphenous **Deep veins** 4. Inferior vena cava 5. Common iliac 6. Internal iliac 7. External iliac 8. Pelvic: gonadal, broad ligament 9. Other common femoral vein 10. Deep femoral vein 11. Popliteal vein 12. Crural: anterior tibia 13. Posterior tibial 14. Peroneal (all paired) 15. Musculargastrocnemial 16. Soleal veins, 17. Other Perforating veins 18. Thigh Calf
C5: healed venous ulcer			
C6: active venous ulcer			
S: symptomatic, ache, pain, tightness, skin irritation, heaviness, and muscle cramps, other complaints attributable to venous dysfunction A: asymptomatic			

Diagram 2(a) CEAP Classification Revision [31]

Note. Only C0 to C4a with leg symptoms are recruited for investigation (C0-C4a/with S)

Clinical Grade	Definition
C0: no visible or palpable signs	**Basic CEAP** Pr: reflux no visible or palpable signs of venous disease
C1: Telangiectases, Reticular veins, malleolar flare or reticular veins	**Basic CEAP** Po: obstruction **Telangiectases** defined by dilated intradermal venules <1 mm diameter **Reticular veins** defined by dilated, nonpalpable, subdermal veins ≤3 mm in diameter
C2: varicose veins	**Basic CEAP** Pr, o: reflux/obstruction Dilated, palpable, subcutaneous veins generally >3 mm in diameter
C3: edema	**Basic CEAP** Pn: no identifiable venous pathophysiology Edema without skin changes
C4 **C4a:** pigmentation or eczema **C4b:** lipodermatosclerosis or atrophie blanche	**Superficial Veins** Skin changes ascribed to venous disease Pigmentation, venous eczema, or both Lipodermatosclerosis, atrophie blanche, or both
C5: healed venous ulcer	Skin changes with healed ulceratuion
C6: active venous ulcer	Skin changes with active ulceration

Diagram 2 (b) CEAP Classification Revision adapted as shortcut [45,46]

Note. Only C0 to C4a with leg symptoms are recruited for investigation (C0-C4a/with S), adapted from Bergan JJ et al. (2006)

2.1.3 Physical Examination, Diagnosis and Management [44-50]

General physical examinations together with extensive family history are essential to direct appropriate diagnosis of CVD. These examinations may demand certain noninvasive testing. However, for differential diagnosis, certain complicate invasive procedures may also be demanded to secure correct diagnosis which are generally

required for both assessing severity of disease or if necessary for surgical intervention. Below were general procedures employed in general practice to assess CVD.

2.1.3.1 Physical Examination [44-49]

The physical examination in terms of physical signs and visual inspection and palpation could provide first signs evidence of chronic venous disorders. The skin is assessed for any outstanding dilated superficial venous abnormalities findings such as telangiectasis, reticular veins and or presence of varicose veins. The skin surface is carefully examined for any irregularities or bulges to suggest if any presence of dilated tortuous veins. The distributions of varicose veins may follow the course of the affected superficial vein and small saphenous veins. The examination may also include positioning both during an upright posture and lying position to allow for maximal distention of the veins. Others skin findings for example hyperpigmentation, stasis dermatitis, atrophie blanche (or white scarring with a paucity of capillaries), or lipo-dermato-sclerosis may be examined. The presence of edematous signs and severity are also important to be assessed. The edematous state due to CVD and chronic venous insufficiency is dependent and usually pitting; Nevertheless, these edematous presentations become more resilient to palpation if protracted. An early finding of venous congestion includes calf fullness or increased limb girth, so the calf muscle consistency should be assessed, and measurement of the limb girth should be performed. There is no universally agreed on scale for grading the severity of edema. The objective examination employing venous clinical severity score (VCSS)

grading of various seven dimensions [32,33] on a basis of level of the most proximal involvement in the limb are essential to allow proper follow-up if any prognosis especially in due course of treatment. The test involves applying a tourniquet or manual compression over the superficial veins after the patient lies down to empty the veins. Thereafter these veins are observed with resumption of an upright posture; in the presence of superficial reflux, the varicose veins will take >20 seconds to dilate; in contrast, in the presence of deep (or combined) venous reflux, the varicose veins will rapidly dilate. In some cases, continuous-wave Doppler, known as venous duplex as a noninvasive test may also be employed in the physical evaluation.

2.1.3.2 Differential Diagnosis [50,51]

A broader differential diagnosis for the most common symptoms of leg swelling and discomfort that are observed in severe stage of CVD could be differentiated with further examination. Basically, it is essential to exclude an acute venous problem, such as deep vein thrombosis. Then, further consideration of (if there are any) systemic causes of edema, such state of cardiovascular system failure leading to heart failure, nephrosis, liver disease, or endocrine disorders. In addition, certain adverse effects of medication especially calcium channel blockers, nonsteroidal anti-inflammatory drugs or oral anti-hypoglycemic agents which should be considered since these agents may bring about edema in the long-term treatment of chronic disease. A special consideration should be aware of any critical disorders such as state of lymphedema, lipedema, and their combination. It is important since the

state of lymphedema cause obstruction of lymphatic drainage which ultimately leads to fluid accumulation especially in the lower limbs which could extend to foot and toes. The impact of CVD which leading to venous insufficiency (CVI) is less likely to impact the foot edema. The edema due CVD and CVI may be pitting in the early stage and later in the courses of the disease, subsequently as disease progresses the skin shall become non-pitting. In the contrary, state of lipedema characterized by accumulations of fatty tissue rather than water fluid, thus, it is a non-pitting and less likely to get involved with feet but rather, often with a cuff of tissue at the ankle. Lastly, additional regional considerations need to be confirmed, especially if there is any ruptured popliteal cyst, exertional compartment syndrome, soft tissue hematoma or mass, or gastrocnemius tear. These uses of extensive assessment findings and other noninvasive testing should allow for the proper diagnosis which need to further secure.

2.1.3.3 Differential Diagnosis with other noninvasive testing

2.1.3.3.1 Venous Duplex Imaging [52-57]

The application of Venous duplex imaging technology, currently is the most common technique used to confirm the diagnosis of CVI and assess its etiology and anatomy. This technique is highly recommended in the Clinical Practice Guideline (grade 1A evidence level). Venous duplex imaging usually combines a B-mode imaging of deep and superficial veins with pulsed Doppler assessment of flow direction with provocative maneuvers. The presence of venous obstruction because of chronic

deep vein thrombosis or venous stenosis could be directly visualized or inferred from alteration in the spontaneous flow characteristics. The direction of flow could be assessed in reverse Trendelenburg position during a Valsalva maneuver or after augmenting flow with limb compression. However, to assess for any reflux involves the use of a cuff inflation-deflation technique with rapid cuff deflation in the standing position. This provides information about the anatomic distribution of reflux disease involving the deep and superficial venous systems and if any perforator veins. The presence of reflux is determined by the direction of flow, because any significant flow toward the feet is suggestive of reflux (given in **diagram 3**). The duration of reflux is known as the reflux time. A reflux time of >0.5 seconds for superficial veins and 1.0 second for deep veins is typically used to diagnose the presence of reflux. A longer duration of reflux implies more severe disease but does not correlate well with clinical manifestations as such in mild and moderate staging of CVD such as C0 to C4, with mild to moderate staging, there is less supporting data to suggest application of Duplex scan in evaluation of clinical manifestation rather than the Venous clinical severity score [53-55]. The venous duplex imaging provides information about local valve function to construct an anatomic map of disease in terms of the systems and levels of involvement. This is often sufficient data to help guide therapy, but if the contribution of the reflux to global hemodynamics is required, then further testing, such as plethysmographic techniques, should be considered.

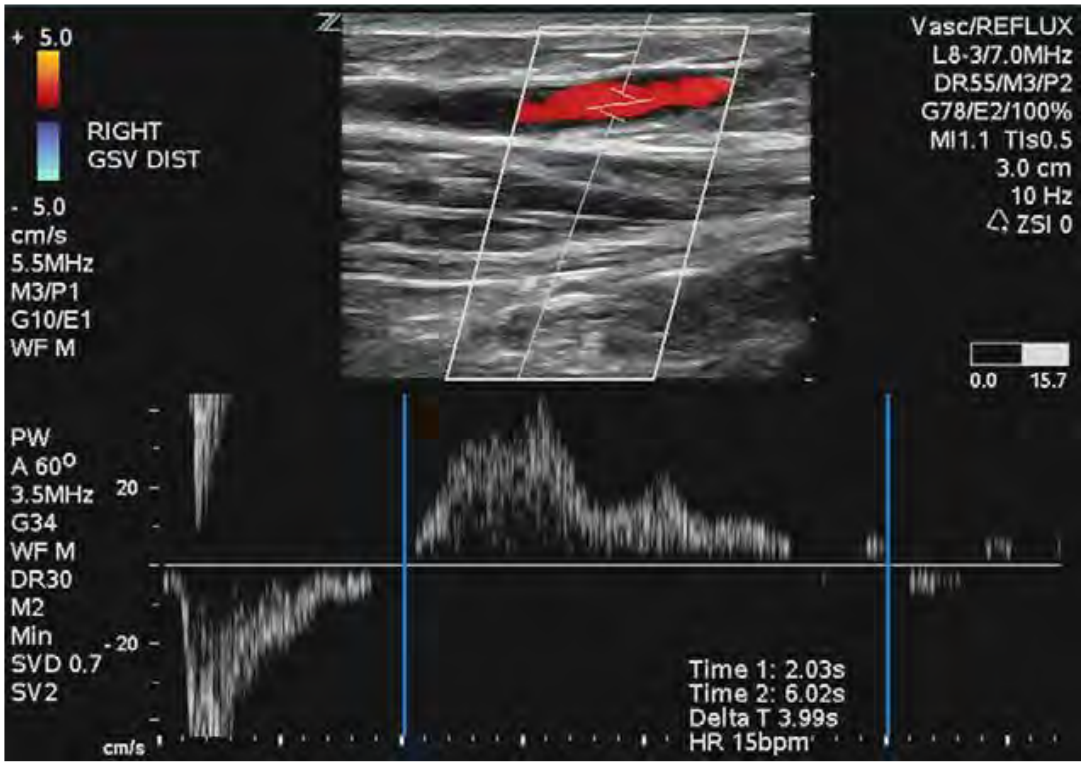


Diagram 3 Venous duplex ultrasound demonstrating reflux in the great saphenous vein.

Blood flow direction is determined after increasing central venous return with rapid cuff inflation then deflation. Flow in the direction of the feet is because of incompetent valves, as shown in red in the color image and above the baseline in the pulse Doppler. The Doppler spectrum quantifies the duration of reflux, and in the example above it is≈ 4 seconds **[**52-57**].**

2.1.3.3.2 Air Plethysmography [58-63,68]

Air plethysmography (APG) technology allows a proper measure in terms of volume of changes for each potential component of the pathophysiologic mechanisms of CVI, including reflux, obstruction, and if there is muscle pump dysfunction. These changes in limb volume are measured by air displacement in a cuff surrounding the calf during maneuvers to empty and fill the venous system (as given in **diagram 4**).

Venous outflow is assessed during rapid cuff deflation on an elevated limb that has a proximal venous occlusion cuff applied. The venous outflow at 1 second, expressed as a percentage of the total venous volume, is used to evaluate the adequacy of outflow. The limb is then placed in the dependent position to evaluate venous filling. The rate of refill, expressed as the venous filling index, is used to determine the presence and severity of reflux. A normal venous filling index is <2 mL/s, whereas higher levels (>4 mL/s) are abnormal. Abnormal venous filling indices have excellent test characteristics to detect reflux (with sensitivity of 70% to 80% and positive predictive value of 99%) and have been found to correlate with the severity of CVI. The function of the calf muscle pump in ejecting blood is determined after 1 and 10 repetitive contractions during toe raises. The volume of blood ejected with 1 tiptoe maneuver divided by the venous volume is called the ejection fraction. Complications of CVI, including ulceration, have been shown to correlate with the severity of venous disease assessed with the venous filling index and ejection capacity. This technique provides quantitative information about several aspects of global venous function and may be used in the selection of intervention and assessment of the response to intervention. APG may be clinically useful when the venous duplex does not provide definitive information on the pathophysiology of CVI, especially in C3 through C6, as recommended in the Clinical Practice Guideline (grade 1B evidence level).

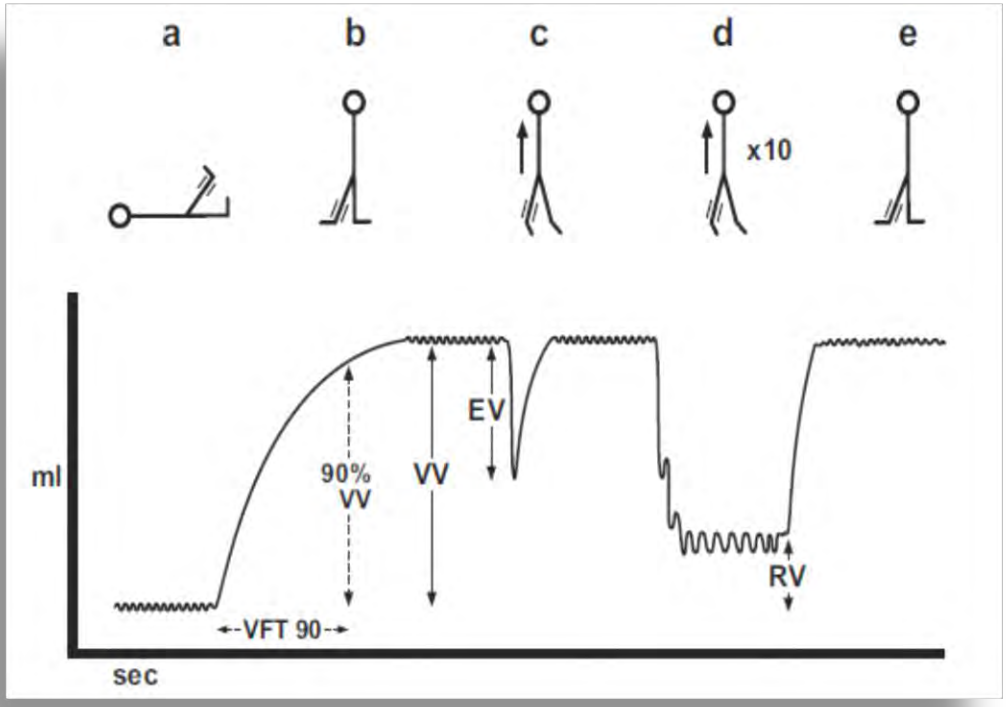


Diagram 4 Air plethysmography (APG) measure changes in venous volume of the lower extremity over time. The ejection fraction (**EF**) is the amount of ejected volume (**EV**) after 1 tiptoe over the total venous volume (**VV**). The EF is a measure of the calf muscle pump function. The residual volume fraction (**RVF**) is the residual volume (**RV**) after 10 tiptoes over the venous volume (**VV**) and is the noninvasive measure of ambulatory venous pressure. The venous filling index (**VFI**) is 90% of the venous volume (**VV90%**) that fills within the venous filling time (**VFT90**) at 90% of the VV. The **VFI** measures venous valvular function and severity of global reflux **[**58-63,68**]**.

2.1.3.3.3 Computed Tomographic or Magnetic Resonance Venography [64-68]

Another advances imaging technology with computed tomography and magnetic resonance have allowed clinical evaluation of venous disease. The technology is the most useful to evaluate more proximal veins and surrounding

structures to assess for intrinsic obstruction or extrinsic compression. Optimal imaging of the venous system requires the use of intravenous contrast material, with appropriate timing of image acquisition in terms of venous filling to obtain a venogram (as given in **diagram 5**). Proper technique is required to avoid artifacts with inadequate venous opacification, with refinements allowing for better visualization to assess for obstructive disease, varicose veins, perforating veins, and other venous malformations. Both computed tomography and magnetic resonance venography may be used to define complex venous anatomy, such as ileo-femoral venous obstruction, before intervention as recommended in the Clinical Practice Guideline (grade 1B evidence level).

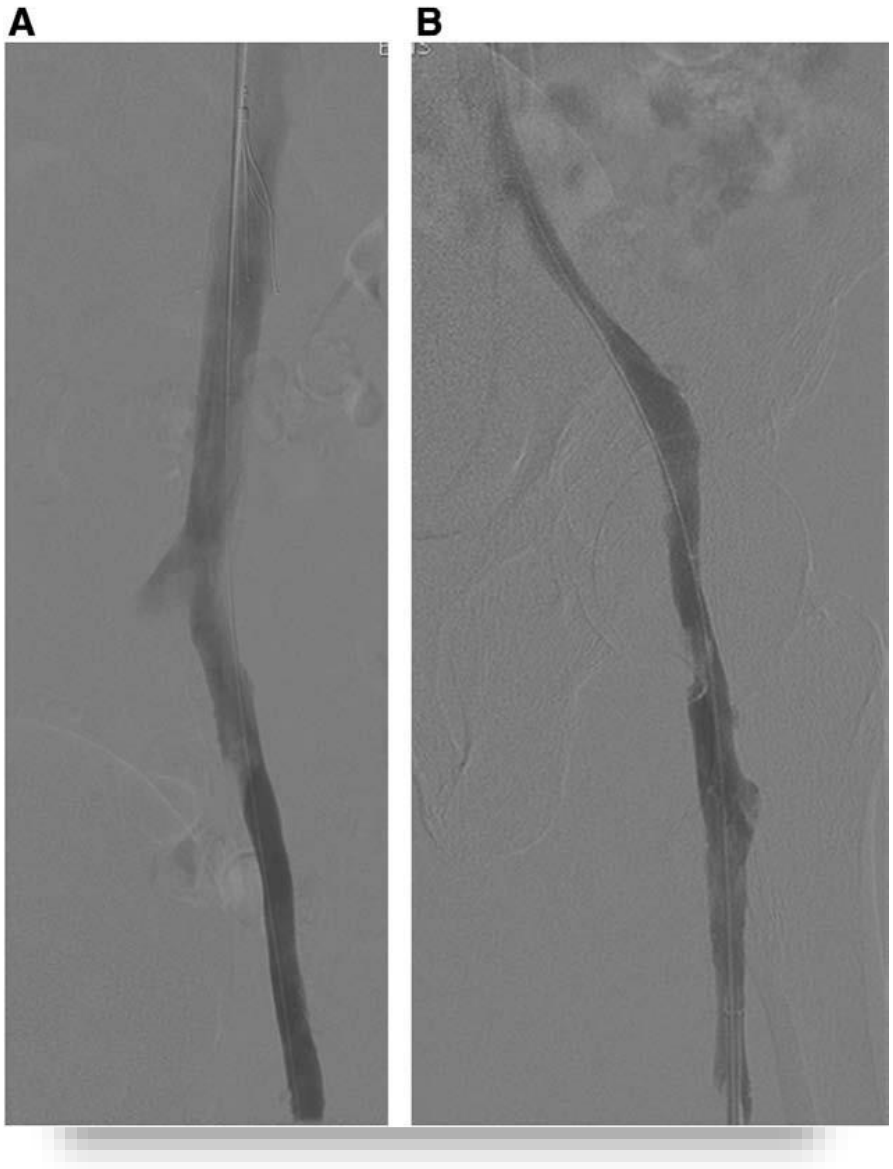


Diagram 5. Venography and Contrast Venography**.**

A, Venography of the ileo-caval segment to assess for patency.

B, Descendings venography of the left lower extremity demonstrating reflux into the femoral vein in a post-thrombotic vein [64-67].

2.1.3.3.4 Other Techniques [68-70]

Other non-invasive technology, such as photoplethysmography, strain gauge plethysmography, and foot volumetry could also be employed. These technologies

provide information with respect to generalized global venous function. The Photoplethysmography may be used to assess time required to refill the veins within the dermis or the venous refill time. It is one of the most useful to determine the absence or presence of reflux disease, however, this is poorly correlates with disease severity.

2.1.3.4 Differential Diagnosis with invasive Testing

2.1.3.4.1 Contrast Venography [64,65,67-70]

The venography technology provides direct physical views of the venous system which can be performed by both an ascending and descending approaches (as given in the **diagram 5**). The ascending approach venography is performed by injection of contrast media into the dorsum of the foot and thereby allow visualization of contrast-traveling cephalad inside the deep venous system of the limb. The technique is very useful to provide details of venous anatomy and thus helpful for surgical procedure. The venography technology is also able to distinguish cause whether it is a primary from secondary. The descending approach venography is performed by proximal injection of contrast in a semi-vertical posture on a tilt table with some aids of the Valsalva maneuver. The technique is very useful to identify reflux in the common femoral vein and at the sapheno-femoral junction as well as other locations

2.1.3.4.2 Intravascular Ultrasound [71-73]

An intravascular ultrasound technology is another popular technique in the management of venous disease and is very helpful to help surgeon to guide interventions (as given in the **diagram 6**). This technique is performed by insertion of a catheter-based ultrasound probe to assess and visualize periluminal vascular anatomy for any obstructive or stenotic disease of the venous system. The intravascular ultrasound techniques are better than venography in giving estimates of venous morphology and of different severity of central venous stenosis.

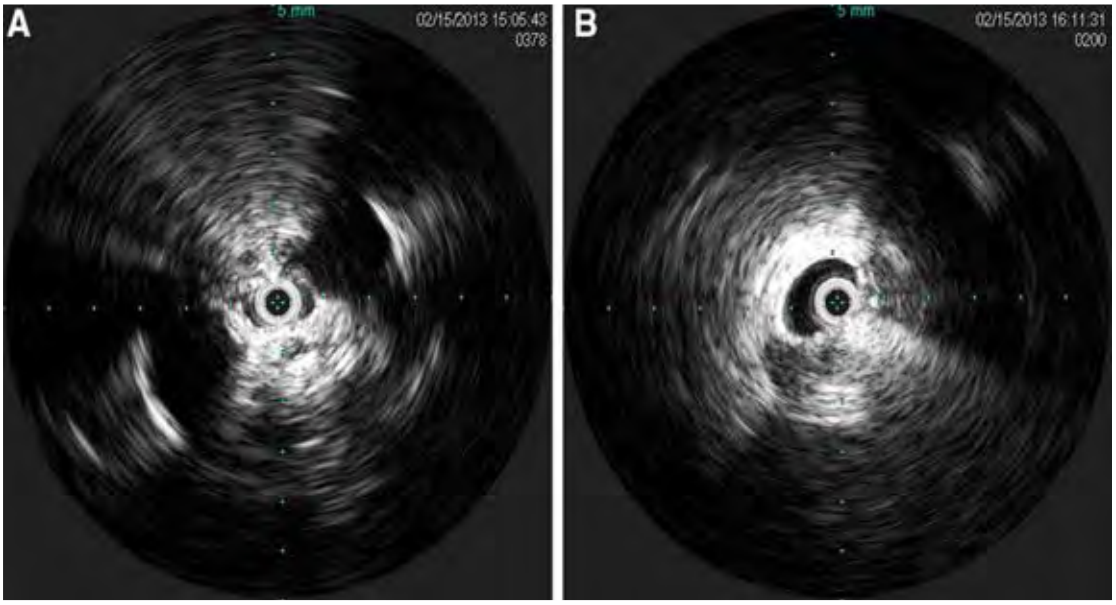


Diagram 6 Intravascular Ultrasound

Intravascular ultrasound of compressed left common iliac vein with minimal lumen diameter. **B**, After Angioplasty and stent of the left common iliac vein, there is significant improvement of the lumen. The hyperechoic Doppler spectra in both images is indicative of severe chronic fibrosis of the vein wall [71-73].

2.1.3.4.3 Ambulatory Venous Pressure [64-68,69,71,73]

Ambulatory venous pressure monitoring is the gold standard in assessing the hemodynamics of CVI. The technique is performed by insertion of a needle into the dorsal foot vein with connection to a pressure transducer. The pressure is determined in the upright posture at rest and after exercise, such as during toe raises. The pressure is also monitored before and after placement of an ankle cuff to help distinguish deep from superficial reflux. Ambulatory venous pressure has been shown to be valuable in assessing the severity and clinical outcomes in CVI. The mean ambulatory venous pressure and refill time are the most useful parameters. This technique provides information on global competence of the venous system. However, there is some concern that this pressure may not accurately reflect the pressure within the deep system. This technique is seldom used in clinical practice because of its invasive nature, potential limitations, and alternative diagnostic modalities.

2.2 CONSERVATIVE MANAGEMENT [76-89]

The aims of conservative management are to reduce aggravating symptoms and prevent development of complications as well as delay disease progression. Compressive stockings are major tools employed for conservative management and if failure, then specific treatment should be used on basis of anatomic and pathophysiologic features of affected leg (as given in the **diagram 7**). Compression

therapy is considered the first-line therapy for those with symptomatic varicose veins or greater (grade 2C evidence level) but not in candidates for great saphenous ablation. Compression therapy is recommended for both patients with venous ulcers (grade 1B evidence level) and as an adjunct to superficial venous ablation to reduce the risk of ulcer recurrence (grade1A evidence level). The prescription for elastic compression stockings in CVI includes information about the tension and length. The tensions are based on the clinical severity with 20 to 30 mm Hg for CEAP class C2 to C3, 30 to 40 mm Hg for CEAP class C4 to C6, and 40 to 50 mm Hg for recurrent ulcers. The most common- length stockings are knee length, because patient adherence is greater and symptom relief adequate. Stockings need to be changed every 6 to 9 months if worn daily with an alternate pair to avoid loss of the tension that the stocking exerts. There are many types of compression such as graded external compression (both elastic or non-elastic, adjustable compression layered bandaging) which aims to oppose hydrostatic forces of venous hypertension. In general, compressive stockings (between 20 and 50 mm Hg of tension) could help relief pain, however patient's noncompliance and long-term cost remain issues. Patients in severe case with the CEAP classes C4 to C6 may require invasive treatment and may need referral to a vascular specialist. Patients education such as maintaining healthy lifestyle behavior such as weight reduction if overweight may improve manifestations of CVD. It is suggested that hemodynamic benefits of compression therapy in patients with CVI have been shown to reduce the residual

volume fraction, which is indicated improving calf muscle pump function, and thus reduce reflux in vein segments. Nevertheless, reverse compression (anti-graduated stockings or progressive elastic compression with higher pressure in the calf than the ankle), found improved calf muscle pump function on ambulation and should reduce edema in severe CVD. In some cases, with severe CVI, it is important to prevent further infection and maintain skin health to compromised skin integrity [83-90]. The topical application of localized moisturizers such as beeswax for dry skin to reduce skin fissuring. In certain stages of CVI where development of stasis dermatitis occur the application of topical steroid could be helpful. Skin cleanliness is required to prevent bacterial overgrowth especially if such skin breakdown and complicated with venous ulcers. In this case, specialized wound cares are demanded to reduce any possible infectious and severe complications. In case of venous ulcer, hydrocolloids, foam dressings are helpful to control wound fluid drainage and maceration of the adjacent skin. Formulation containing silver impregnated dressings have been employed to control infection and restore tissue integrity for infected ulcers, however, this remains controversial. Biologic skin substitutes and tissue engineering products have been recommended for difficult-to-heal venous ulcers.

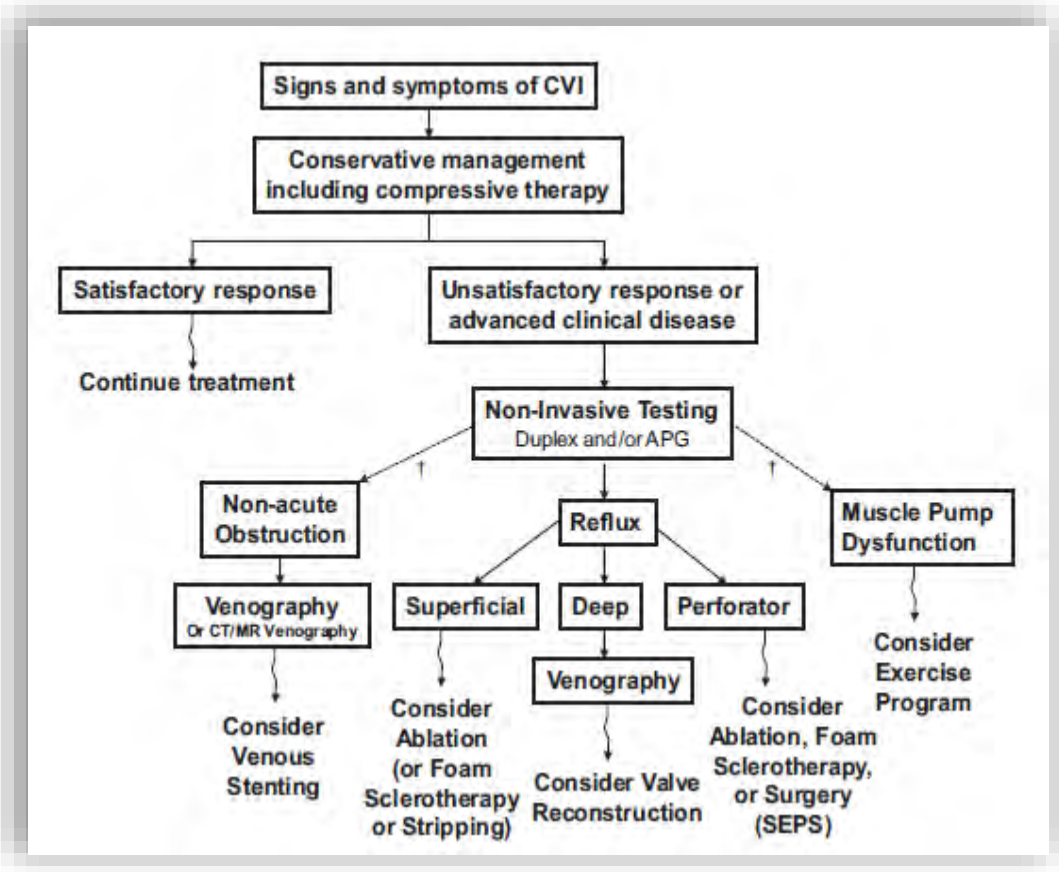


Diagram 7. Specific treatment plans should be observed respecting the anatomic and pathophysiologic features of affected leg: General view for diagnosis and treatment of chronic venous insufficiency (CVI) with respects to pathophysiologic mechanism. Multiple pathophysiologic mechanisms within the same require a combination of treatment options.

2.3 MEDICAL MANAGEMENT [90-98]

Variety of oral medications both modern synthetic drugs and herbal-derived plants extracts have been investigated in the treatment of CVI as following: (1) Coumarins (α-benzopyrenes) to prevent thrombosis, (2) Flavonoids (γ-benzopyrenes) to prevent vascular inflammation and reduced edematous state, (3) Saponosides (horse

chestnut extracts) to prevent inflammation, and (4) Variety of claims from other plant extracts in anecdotal reports. All the afore-mentioned drugs were primarily classified as so-called veno-active drugs one way or another that may render pharmacologic activity on veins though have not been standardized or gain recognition as standard treatment of CVI. However, precise mechanism of action for these drugs have not been fully investigated or medically confirmed. Their possible multiple mechanisms may probably explain effectiveness as comparable to compression stockings in the short term. Nevertheless, there is no conclusive or definitive evidence to support for long-term beneficial effects should patients taken the medication orally at reducing leg edema and pain from CVI, especially against its questionable long-term safety. Though, oral aspirin had been reported to promote venous ulcer healing, prevent recurrence of venous ulceration and recurrent thromboembolic events in those with unprovoked thrombosis. There are certain systemic adverse reactions which could be shortcomings of aspirin despite its clinical benefits. However, so far there is no clinical investigation of aspirin efficacy in mild to moderate chronic venous disease [97-100]. As above, at present, there is no standard medical treatment practically available for patients suffering from leg symptoms due to chronic venous disease despite symptomatic relief.

2.4 OTHER INTERVENTIONAL MANAGEMENT [100-113]

2.4.1 Sclerotherapy [74,100,106]

The venous sclerotherapy is an interventional procedure to obliterate telangiectases, varicose veins, and other venous segments. The venous sclerotherapy employs the treatment with sclerosing agents, or may be used in conjunction with other surgical procedures to correct the on-going progression of disease vein. These attempts by sclerotherapy are suitable for some venous conditions and location such as for (1) small size spider veins (less than 1 mm in diameter), (2) small size varicose veins ranges between 1- to 4-mm in diameter with venous lake, (3) any bleeding due to varicosities, and (4) malformation of the small vasculartur e. Several types of sclerosing agents could be used such as (a) sodium chloride hypertonic concentration, (b) detergents such as sodium tetradecyl sulfate, sodium morrhuate, sodium iodide, polidocarnol and chromated glycerin.

2.4.2 Endovenous Ablative Therapy [101-103]

The procedures employ heat or thermal energy derived from radiofrequency where laser was used to ablate the problematic veins. The heat generated causes local thermal injury to the vein wall which on the contrary could lead to thrombosis and fibrosis. The radiofrequency ablation of problematic veins may not be able to provide complete obliteration, mostly only among 85% of patients after 2 years may need further recanalization. The success of endovascular ablative therapy depends on the type of procedures and size of veins. Both radiofrequency and laser

treatment are performed with tumescent anesthesia to prevent skin burns and reduce pain. After the procedures, patients may return to normal activities. There are some shortcomings as potential complication of ablation such as deep venous thrombosis and pulmonary embolism. Several studies comparing endovenous ablation with conventional ligation and surgical stripping has founded that their short-term efficacy and safety are comparable. Only with endovascular ablation, patients can quickly recover better quality of life and allow them to return normal activity but not for surgical stripping. Some new ablative technologies are invented and could avoid tumescent anesthesia to reduce pain and nerve injuries has been tests and later to come into the market.

2.4.3 Endovenous Deep System Therapy [103-106]

Endovenous deep system therapy is the technique to bring back venous outflow and help reshaping obstruction of deep veins through stenting. The endovenous deep system employ stenting known as venous stenting on problematic deep veins, recent studies supported that iliac vein stenting produced success response with clinical outcomes especially in terms of relief of pain and complete healing of ulcer over half of patients treated. The same success in completed elimination of edema among over one third of patients treated also cited. However, patency of venous stents appears to be durable for at least 3-6 years. It is therefore demand a close follow-up to ensure should stent patency maintained especially in high risk patients.

2.5 SURGICAL MANAGEMENT [107-111]

The surgical approach in management of chronic venous insufficiency at the end of the day is demanding to complement the treatment refractory cases from compressive stocking, medical and endovenous therapy. The surgical procedures include patients with persistent pain or discomfort irrespective of staging. The surgical mean aims primarily to relief overall disability and failure from others procedure especially for the difficult to heal venous ulcers. Surgical mean is only optional in certain patient whom condition failure to respond compression stocking or due to veins varicosities recurrence are main problems. Surgery approach requires special consideration for success with respect to site and type of defected veins. For the truncal veins, it is target to prevent consequences of reflux in the superficial venous system by either interruption and/or removal. For the tributaries veins, the surgery is target to remove varicose veins. Excision, ligation and stripping are surgical procedures successful to bring back an improved venous hemodynamics, provide symptoms relief and healing of venous ulcers. The surgical approaches are ultimate procedures to repair the perforated veins, improve and repair venous valve injury that lead to venous dysfunction and further development and progression of chronic venous insufficiency. The surgical approach is indicated to all CEAP clinical class should it refractory to other management.

2.6 HERBAL MEDICINE AND CHRONIC VENOUS DISEASE [110-121]

A basic literature search through electronic databases PubMed, Medline and the Cochrane Library was initiated. The search query began with “drug treatment of chronic venous disease” filtered with subjected to clinical trial, available as at least abstracts and investigation only in human since 1960 to 2015. There were only 356 articles founded. Subsequently to screening through each of the abstracts to exclude the overlapping CVI reported within either the domain of other cardiovascular disease or CVI mimic with venous leg ulcers. Only limited drug options were available for treatment of CVI such as Titrated Extract of Centella Asiatica (TECA), Horse-Chestnut Seed Extract (HCSE), Extract of a French Maritime Pine Bark (Pycnogel®), Micronized Purified Flavonoid Fraction (MPFF), Ginkor Fort, Buckwheat Herb Tea, Red-Vine-Leaf Extract (RVLE), Chinese herbal medicine and Acetyl Salicylic Acid (ASA). Exhaustive scientific search employing EndNote with subject to search terms for the combination of intervention and disease designation as follows “Intervention: Herbal plants, herbal extracts, herbal medicine, herbal products, traditional Chinese medicine, TCM” and “Designated disease: Chronic Venous Disease, Chronic Venous Insufficiency, Venous Disease, Venous Insufficiency” which also employs the search engines from PubMed database, EBSCO, Google Scholar, these results are summary as follows:

Ernst E., 1999 reviewed herbal treatments for common illness in the elderly and had reported that Aesculus hippocastanum (horse chestnut) seed extracts could alleviate subjective symptoms and reduce the objective signs of chronic venous insufficiency. The author commented on studies which also suggested further investigation [110].

Kiesewetter, H et al, 2000 conducted a clinical investigation looking at the efficacy of Red vine leaf extract (RVLE), as herbal medicine containing many types of flavonoids, quercetin-3-O-beta-glucuronide and isoquercitrin (quercetin-3-O-beta-glycoside), in both stage I and II chronic venous disease patients. The study was conducted for 12 weeks, as a randomized, double-blind, placebo-controlled, parallel-group, multi-center study. A once-daily doses of 360 and 720 mg RVLE (pharmaceutical extract code AS195; Antistax Venenkapseln) was randomized as compared with placebo to assess the efficacy and safety of in patients with stage I and incipient stage II chronic venous insufficiency. All recruited CVD patients were in ambulatory setting between 25 to 75 years old and diagnosed with stage I & II CVD, without extensive trophic changes, including not having other complicated medical conditions, not being treated with compression stockings or not currently receiving diuretics and drugs affecting fluid imbalance. Patients were randomly assigned to a double-blind treatment with placebo, 360 mg AS195 or 720 mg AS195 once daily for 12 weeks. At the screening, a run-in period, all patients received a single-blind 2-week placebo run-in as baseline, followed by double-blind randomization for 12 weeks and then followed by a single-blind at the end of randomization for 2-week

placebo washout. Evaluation criteria performed at baseline, after 6 and 12 weeks of treatment and 2 weeks after discontinuation of treatment. It was found that, out of 260 patients enrolled and randomized, only 219 completed the study in accordance with the protocol. Analysis as in the intention-to-treat analysis, there were 257 subjects, reported that lower leg volume in mean ±SD (measured by water displacement plethysmography) , the patients treated with placebo (N = 87) had lower leg volume increased by 15.2 +/- 90.1 and by 33.7 +/- 96.1 g after 6 and 12 weeks compared to baseline. Patients treated with AS195 had lower leg volume decreased, and after 12 weeks of treatment, the mean lower leg volume treated by active treatments as compared with placebo were reduced by -75.9 g (95% CI: -106.1 to -45.8 g) for a group treated with 360-mg AS195 (N = 86), whereas reduced by -99.9 g (95% CI: -130.3 to -69.6 g) for a group treated with 720-mg AS195 (N = 84) respectively. Treatment with the lower doses 360 mg AS195 reduced calf circumference less than treated with higher doses 720 AS195. All both active treatments provided benefits in clear reduction in circumference over time as compared with placebo (95% CI of the estimated treatment effects vs. placebo after 12 weeks: -1.40 to -0.56 cm and -1.73 to -0.88 cm for lower and higher doses, with $p<0.001$ respectively). Moreover, subjective symptoms improvement was well observed at 6 weeks with both active and placebo treatments. However, further improvement at week 12 observed only in the active treatment. Overall, after 12-week treatment, the subjective symptoms improvements were significantly better as

compared with placebo from baseline with p< 0.001. The treatments were safe, well tolerated with rare mild adverse events. Nevertheless, two adverse events (AEs) in the placebo group led to hospitalization. There were, however, an uncertainty of the research finding due basically to in methodology issue which demanded further investigation [111].

Ernst, E et al, 2002 conducted a critical review of evidence related to complementary and alternative medicine use for dermatologists in clinical practice by systematic literature searches from Medline, EMBASE, The Cochrane Library, CISCOM and AMED (until October 2000) of the published literature based on systematic reviews and randomized controlled trials with two conditions (atopic dermatitis and chronic venous insufficiency) and two treatment modalities (Aloe vera gel and tea tree oil). For chronic venous insufficiency, there were evidences for the effectiveness of oral horse chestnut seed extract and for Aloe vera gel. The authors also suggested the use of complementary and alternative medicine as systematic treatments possess some risks [112].

Rohdewald, P et al, 2002, conducted a review of studies with both Pygnogenol (PYC) and its possible mechanisms for activity in men. The reviews performed by selecting studies both from peer reviewed literature, unpublished data presented at international meetings and other data from published sources in German and French languages. The authors proposed that PYC was primarily composed of procyanidins and phenolic acids. PYC contains a wide variety of procyanidins that range from the

monomeric catechin and taxifolin to oligomers with 7 or more flavonoid subunits. The phenolic acids are derivatives of benzoic and cinnamic acids. The ferulic acid and taxifolin components are rapidly absorbed and excreted as glucuronides or sulphates in men, whereas procyanidins are absorbed slowly and metabolized to valerolactones which are excreted as glucuronides. PYC has low acute and chronic toxicity with mild unwanted effects occurring in a small percentage of patients following oral administration. The author also reported that PYC is effective in the treatment of chronic venous insufficiency and retinal micro-hemorrhages by (1) its anti-oxidative stress activity reported in in-vivo models such as a UV-radiation-induced erythema model. PYC antagonized the vasoconstriction caused by epinephrine and norepinephrine by (2) increasing the activity of endothelial nitric oxide synthase and by (3) dilation of the small blood vessels has been observed in patients with cardiovascular disease. PYC prevents smoking-induced by platelet aggregation and by (4) reduces concentration of thromboxane. PYC relieves premenstrual symptoms, including abdominal pain and this action may be associated with the spasmolytic action of some phenolic acids. The author proposed that PYC has beneficial effects on physiological functions that may be effective in chronic venous disease [113].

Bylka, Wieslawa, et al 2005. reported that some extracts of the plants such as horse-chestnut seed extracts and other natural compounds such as aescin, rutin, troxerutin, diosmin and hesperidine acts on the venous system. Rusci aculeati

rhizome was effective on the symptoms of chronic venous insufficiency and hemorrhoids. These plants-derived have long tradition in herbal medicine for their venotonic and anti-edematous properties which were all deserved for further investigation [114].

Lim, Kar Seng et al, 2007, studied the use of Topical Traditional Chinese Medicaments (TTCM). TTCM could be contact sensitizers in patients with chronic venous leg ulcers and its impact in the clinical management of these patients. They used patch-test to the TTCM series. They were also patch-tested for other allergens from the Standard Series, Medicament Series, Steroid Series and wound dressings. All of 44 patients were patch-tested, 17 of the 44 (38.7%) patients were using or had used at least 1 TTCM. Seven patients (15.9%) had at least 1 positive patch-test (PT) reading to TTCM, giving a sensitization rate of 41% (7 out of 17). A significantly high proportion of the patients, 94.1% (16 of 17) with a positive history of TTCM usage had at least 1 positive PT reading as compared to those without a history of TTCM usage, 45.8% (11 of 24). TTCM is an important element and may be cause as contact sensitizers in patients with chronic venous leg ulcers, a significant factor in non- or poor-healing leg ulcers [115]. **Felixsson, Emma et al 2010.,** investigated effects of extracts from seeds and bark of horse chestnut (*Aesculus hippocastanum L*) as an herbal medicine against chronic venous insufficiency. The aim of this study was to observe the mechanisms of action of horse chestnut on the contraction of bovine mesenteric veins and arteries, and human platelet aggregation. It was

founded that horse chestnut extract dose-dependently contracted both veins and arteries, but more effects on veins. These were significantly inhibited by the 5-HT-2A receptor antagonist Ketanserin but not cyclooxygenase inhibitor indomethacin, the alpha-1 receptor antagonist Prazosin or the angiotensin AT-1 receptor antagonist Saralasin. Moreover, the ADP-induced human platelet aggregation was significantly reduced by horse chestnut even with presence of the inhibitor Ketanserin. The authors concluded that horse chestnut mediated vascular contraction through 5-HT-2A receptors, and caused human platelet aggregation [116].

Pawlaczyk, Izabela et al, 2010 used an acidic glycoconjugate from Lythrum salicaria L. with thought that this was effective on hemostasis as used in traditional medicine and pharmaceuticals internally in a form of decoctions or as extracts for treatment of diarrhea, chronic intestinal catarrhs, hemorrhoids and eczema, or externally to treat varicose veins, venous insufficiency and gums. The authors isolated the plant glycoconjugate from flowering parts of Lythrum salicaria, and tested its effects on blood coagulation process. The air-dried flowering parts of this plant a water-soluble glycoconjugate has been isolated by hot alkaline extraction followed by neutralization and purification by multi-steps extraction with organic solvents, dialysis and concentration and tested in vitro on anticoagulant activity on human plasma, and on Wistar rats blood system in vivo as well as ex vivo. In vitro and ex vivo experiments showed complete inhibition of plasma clot formation traditional use of Lythrum salicaria as a pro-coagulant activity [117].

Wei, XL et al, 2011., conducted investigation to test clinical efficacy of Mailuo Shutong Granule, a compound traditional Chinese herbal medicine, and Hirudoid cream (heparinoid), in treatment chronic venous disease among 180 out-patients diagnosed with dermal hyperpigmentation due to skin hyperpigmentation at vascular surgery clinics. All patients were randomized into three groups with 36 patients each as (1) Mailuo Shutong (2) Hirudoid and (3) A combined therapy groups. The treatments were only a short-term with continue treatment for 28 days. The study reported only results of area of pigmentation decreased including gray value of skin pigmentation as compared before and after treatment ($P<0.05$). The combined group were significant better than either Mailuo Shutong or Hirudoid alone ($P<0.05$). The authors concluded that Mailuo Shutong Granule and Hirudoid cream can improve CVD-induced hyperpigmentation, and suggested that combined treatment of the two gave better clinical outcomes though this research methodology have no control for comparison [118].

Schoones A., et al, 2012, investigated whether Pycnogenol, originally herbal dietary supplement extract from French maritime pine bark extract in a concentrated form with 70% procyanidin antioxidant could be useful for certain chronic disease including chronic venous disease through literature search and analysis from various scientific database such CENTRAL PubMed , MEDLINE and EMBASE and clinical trial registries and other bibliographies manual search form data obtained from manufacturer of Pycnogenol. The specific search criteria included randomized

controlled trials whereas obtained clinical outcomes including all-cause mortality as primary outcomes whereas adverse events and biomarkers of oxidative stress also reported. In the searching methodology for pooled analysis, the following conditions required, (1) two authors independently assessed trial eligibility, extracted all data and assessed risk of bias meanwhile (2) the third author additionally extracted information on outcomes and results. There were 15 trials with 791 patients for treatment of seven different chronic disorders including osteoarthritis (3 studies; N = 293), T2DM (4 studies; N = 201), asthma (2 studies, N = 86), hypertension (2 studies; N = 69) attention deficit hyperactivity disorder (1 study, N = 61), **chronic venous insufficiency (2 studies; N = 60)**, erectile dysfunction (1 study; N = 21). No conclusion could be drawn due to major risk of bias and the analysis generated systematic error [119].

Morling, Joanne et al, 2013, conducted a systematic review to investigate effects of Rutosides, extract from horse chestnut (*Aesculus hippocastanum*) for treating edema formation in chronic venous insufficiency (CVI) in patients diagnosed with Deep Vein Thrombosis (DVT) suffering from pain, swelling, and skin changes in the limb. The analysis included interventions compared purely Rutoside with Placebo as controlled to determine the effectiveness and safety of rutosides, without other added on intervention including elastic compression stockings (ECS) or any other treatments. The analysis and reviews were conducted on behalf of the Cochrane Peripheral Vascular Diseases Group Trials Search Coordinator searched the Specialized Register.

Two authors independently assessed studies for inclusion and extracted data by employing prepared data extraction forms. The analysis also assessed with the risk of bias using Cochrane risk of bias software. The primary outcome measures were the occurrence of leg ulceration over time and any improvement or deterioration of post-thrombotic syndrome. The secondary outcomes included edema reduction, pain symptoms and recurrence of deep venous thrombosis or pulmonary embolism, compliance with therapy, and any adverse effects. The statistical analysis employed the Mantel-Haenzel fixed-effect model odds ratios. There were total of 233 participants met the inclusion criteria. There was no evidence that rutosides were superior to the use of placebo. The authors concluded there is limited evidence to support venoactive remedies including rutosides could significantly reduce symptoms of chronic venous disorders [120].

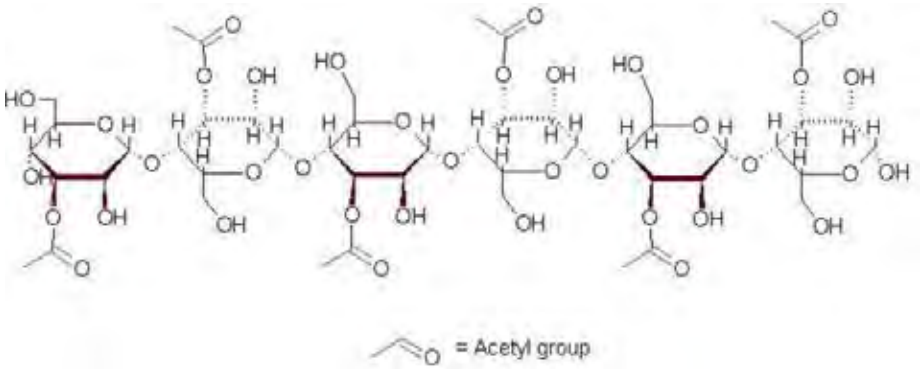
2.7 SELECTED LITERATURE OF HERBAL MEDICINE

2.7.1 Aloe vera: Acemannan [15-22,121-141]

Aloe vera plant has been known for centuries as medicinal plant with its distinct properties. Greek scientists have regarded Aloe vera as “Universal Panacea” whereas “Plant of Immortality” by Egyptians. Aloe vera plant has been employed in cosmetic and food supplement. In addition, Aloe vera has been well known for its application in herbal medicine in United States, European countries, South American countries, India, China, Japan and several countries in Asia. There are approximately 500 species

of Liliaceae family. Each Aloe vera plant has 15 to 30 tapering leaves, each up to 0.5 m long and 8 to 10 cm wide. Beneath the thick cuticle of the epidermis lies the chlorenchyma. Between this layer and the colorless mucilaginous pulp containing 1) clear aloe gel that contains 99% water which is made of glucomannans or acemannan, amino acids, lipids, sterols and vitamins 2) vascular bundles and inner bundle sheath cells which contains a bitter yellow sap exudes when the leaves are cut. This middle layer of latex contains Anthraquinones and glycosides. 3) The outer thick layer of 15–20 cells called as rind which has protective function and could synthesizes proteins and carbohydrates [15].

Acemannan ($C_{66}H_{100}NO_{49}$; Mr 959.15) (IUPAC name: (2S,3S,4R,5S,6S)-6-[(2R,3R,4R,5S,6R) -6-[(2R,3S,4R,5S,6R)-5-acetamido-6-[(2R,3R,4R,5S,6R)-4-acetyloxy-6[(2R,3R,4R,5S,6R)-4-acetyloxy-6-[(2R,3R,4R,5S,6S)-4-acetyloxy-5-hydroxy-2-(hydroxymethyl)-6-methoxyoxan 3-yl]oxy-5-hydroxy -2-(hydroxymethyl)oxan-3-yl]oxy-5-hydroxy-2-(hydroxymethyl)oxan-3-yl]oxy-4-hydroxy-2-(hydroxymethyl)oxan-3-yl]oxy-4-acetyloxy-5-hydroxy-2-(hydroxymethyl)oxan-3-yl]oxy-4-acetyloxy-3-[(2R,3S,4R,5R,6R)-4-acetyloxy-5-[(2R,3S,4R,5R,6R)-4-acetyloxy-3-hydroxy-6-(hydroxymethyl)-5-methoxyoxan-2-yl]oxy-3-hydroxy-6-(hydroxymethyl)oxan-2yl]oxy-5-hydroxyoxane-2-carboxylate)



Aloe vera, as given in Botanical Definition described in WHO Monograph [16], Aloe is dried juice of the leaves of *Aloe vera* (L.) *Burm. f.* or of *A. ferox Mill.* and its hybrids with *A. africana* Mill. and A. *spicata* Baker (Liliaceae). The common synonyms of Aloe are known as following: 1) *A. barbadensis* Mill 2) *A. chinensis* Bak 3) *A. elongata* Murray 4) *A. indica* Royle

5) *A. officinalis* Forsk 6) *A. perfoliata* L 7) *A. rubescens* DC 8) *A. vera* L. var. littoralis König ex Bak. 9) A. vera L. var. chinensis Berger 10) *A. vulgaris* Lam

Aloe contains major and active anthraquinone principles hydroxyanthrone derivatives, mainly of the aloe-emodin-anthrone 10-*C*-glucoside type, known as barbaloin (aloin) and hydroxyaloin which found in the sap. Aloe also contains amino acids, enzymes which have been identified such as amylase, aliiase, bradykin, alkaline phosphatase, carboxylase, catalase, lipase, and peroxidase, minerals, vitamins, lignins, monosaccharide, polysaccharides, salicylic acid, saponins and sterols. Aloe vera contains Aloe gel, the clear mucilaginous part between the green leave and major polysaccharides known as glucomannon or acemannan which are complex

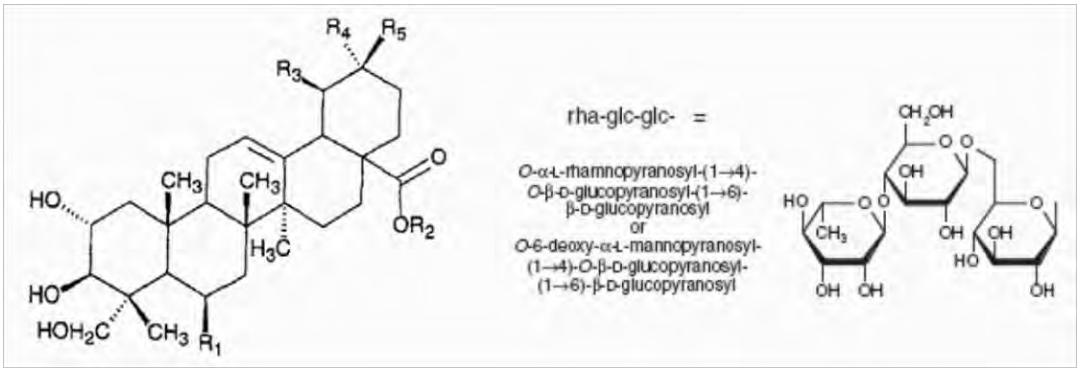
long-chain non-branched polysaccharides with mannose, glucose. In most of commercial extract, a standardized global source, international supply herbal extracts source identified mainly Acemannan as the major active substance demanding most in the extracts. Aloe vera oral administration intended as therapeutic herbal medicine were widely investigated in Diabetese and as Hypoglycemic agents [15,121-126], in Peptic ulcer diseases, ulcerative colitis and irritable bowel syndrome, peptic ulcer as well as in prevention of aspirin induce gastric ulcer and various form of acid peptic diseases [15,21-22,121,127-135], as adjunct treatment of Oral Mucositis in cancer patients [17,137]. Aloe vera external administration has been supported with several successful clinical outcome trials for wounds and ulcers healing which could be summarized as follows: (1) Aloe vera gel dressing accelerated skin healing by approximately 72 hours after dermabrasion as compared with polyethylene oxide gel dressing [18]. (2) Aloe vera gel was effectively accelerated wound healing after frostbite injury, [137,138]. (3) The Vulnopur spray containing Aloe vera was more effective than isotonic saline in wound cleansing for pressure ulcers tested in 133 patients [18-20, 140], (4) Aloe vera cream was superior to placebo report in a placebo-controlled, double-blind trial for treatment of psoriasis (83.3% Vs 6.6%, $p<0.001$) [19,141]. Aloe vera crude extract emulsion could reduce scaliness and pruritus in patients suffering from seborrheic dermatitis [20].

As above, Aloe vera gel-base, with active acemannan have been evidenced for both internal and external use in many therapeutic clinical trials as partly due to its proposed mechanism of action by its anti-inflammation, immune-modulation, anti-bacteria, anti-virus and skin protective. These proposed therapeutic activities are rationale for selection of Acemannan as active substance in the herbal medicine combination formulation, whereby as external formulation 2% acemanan was suggested as base preparation [15,18,121,140].

2.7.2 Titrated Extracts of *Centella asiatica* (TECA): Asiaticoside [23-25,121,142-148]

Centella asiatica (L.) Urban or Hydrocotyle asiatica L is a common scientific name, one of the oldest herbal plants found in many parts of the world especially in many tropical countries. It is a perennial herb creeping plant easily grows in the wet and dam area with rooting at nodes belonging to the plant family Apiaceae(Umbelliferae) which have 20 different species. In the west, it is commonly called as Indian Pennywort (English), in Japan called as Tsubo-kusa (Japanese), and in China called as Tungchian and Luei Gong Gen, in India, known as ‘Indian Pennywort’ “Jal Brahmi” or ‘Mandookaparni’ or Gotu Kola.

As per the European Pharmacopoeia, the standard of herbal *Centella asiatica* contains 6% of total triterpenoid derivatives, called as Asiaticoside ($C_{48}H_{78}O_{19}$; Mr 959.15) (IUPAC name: 6-[[3,4-dihydroxy-6-(hydroxymethyl) -5-(3,4,5-trihydroxy-6-methyl-oxan-2-yl) oxy-oxan-2-yl] oxymethyl] -3,4,5-trihydroxy-oxan-2-yl]10,11-dihydroxy-9-(hydroxymethyl)-1,2,6a,6b,9,12a-hexamethyl 2,3,4,5,6, 6a,7,8,8a,10,11,12,13,14b-tetradecahydro-1H-picene-4a-carboxylate)



Centella asiatica (L.), a well known herbal plant used over 3000 years in by diverse ancient cultures and tribal groups. It is native plants in many countries in the world such as India, Malaysia, Sri Lanka, Madagascar, South Africa, China. In Thailand and Malaysia, *Centella asiatica* has been a common vegetable supplements as food. The fresh water extracts of Centella asiatica have been consumed by Thai, Java, the Malaysia for years as herbal drinks.

Centella asiatica generally contains 0.1 % essential oils, its major component of *Centella asiatica* essential oil is an unidentified terpenic acetate and other compounds such as β-caryophyllene, trans-p-farnesene, germacrene and other

wide ranges ofconstituents such as glycosides (indocentelloside, brahmoside, brahminoside, theankuniside, isothankuniside). Other chemical constituents which had been reported such as vallarine, hydrocotylin, pectic acids, steroids, hersaponin, bacogenin, monnierin, tannins. The raw leaves of *Centella asiatica* are rich in carotenoids, vitamins B and C with flavonoids, castilliferol 1 and castillicetin 2. The flavonoids apigenin, rutin and quercetin have also been detected in methanolic extracts of *Centella asiatica.* Moreover, a polysaccharide isolated from ethanolic known as arabinogalactan (AG) also have been reported. The general herbal preparation of *Centella asiatica* is mostly obtained by alcoholic or aqueous extracts then refined and purified extracts as well as reconcentrated extracts have been have been made commercially available in a wide ranges of global international supply herbal extract sources known as Titrated Extracts *Centella asiatica* (TECA) containing either 40% or 60% of asiaticoside of the aglycons (asiatic acid and madecassic acid). However, in clinical studies, the use of the following commercialized products such as Madecassol®, Blastoestimulina® (titrated extract of *Centella asiatica*) or Centellase® (total triterpenoid fraction of *Centella asiatica*). The most widely used concentration of *Centella asiatica* as extracts is TECA 40% containing 40% of asiaticoside and of the triterpenic genins (asiatic acid and madecassic acid). In general, purification of the herbal extracts involving various steps for chemical treatments are extreme complicated yielding a final combination of refined extract. In the WHO standards, with an isolated constituent and the natural proportion of the

components for *Centella asiatica* is not well maintained. Therefore, based on manufacturing process, though the TECA extract cannot be classified as an herbal preparation due to the manufacturing steps and definitive composition variation, many such clinical testing employed TECA as the main components for formulation and as such the rationale of this herbal extract combination do employ the same as 40% TECA from global supply international standards. The anticipated active constituents of TECA are therefore based on the asiaticoside as madecassic, madecassoside, asiatic acid and asiaticosides which known asto pentacyclic triterpenes [154,155]. The therapeutics activities of *Centella asiatica* varied and reported in many therapeutics area such as for wound healing for the treatment of various skin conditions among leprosy, allergic lupus, varicose ulcers, eczema, psoriasis, diarrhoea, fever, amenorrhea and genitourinary tract infection and urethritis, fever, bronchitis and asthma, relieving anxiety and improving memory and cognition depends on ethanological used in different region. The pharmacologic attributes mainly demonstrated it anti-oxidant and anti-proloferative properties [156,157]. Selected therapeutic effects of *Centella asiatica* on Chronic Venous Insufficiency, varicose vein are as follows:

Pointel JP, et al 1987, conducted a study among 94 patients suffering from venous insufficiency of the lower limbs as a multicenter, double-blind versus placebo study. After randomization, all patients were allocated for a treatment of two months to receive either one of three groups: TECA 120 mg/day, TECA 60 mg/day, or placebo.

The authors reported a significant difference ($p < 0.05$) in favor of TECA for improvement of symptoms of leg heavy, leg edema and satisfactory for overall evaluation by the patient. The venous distensibility measured by a mercury strain gauge plethysmograph at three occlusion pressures was even improved for the TECA groups but in contrary aggravated in placebo treatment group [144].

Cesarone MR et al, 1994, 2001 conducted a prospective, randomized trial looking at efficacy of an oral preparation of Titrated Extracts *Centella asiatica* in the microcirculation and reducing leg edema (leg volume) among fourty patients suffering from venous microangiopathy patients with venous hypertension. Twenty patients for each group was prescribed randomly with either a 60-mg tablet of TECA or placebo, twice daily for 6 weeks. The study was a pre-and-post treatment comparison, after treatment there was a decrease in resting flux (29%) and an improvement (increase) in venoarteriolar response (52%); Partial arterial pressure of oxygen (PO_2) was increased (7.2%) and partial arterial pressure of carbondioxide (PCO_2) decreased (9.6%). There was an important decrease in leg volume (66 mL decrease; 1.3% volume variation). The difference in flux, oxygen and carbondioxide contents (O_2-CO_2) and volume parameters were significant and clinically important at 6 weeks in the active treatment group. In conclusion, the authors reported clinical efficacy of TECA though there was no proper controlled or comparison between placebo and active treatment groups [145,146].

Belcaro G et al, **2001** assessed effects of oral TECA 60-mg tablets taken twice daily for 4 weeks among 50 patients on local capillary filtration with the vacuum suction chamber (VSC) and the rate of ankle swelling (RAS) in patients with ankle edema due to venous hypertension before and after treatment. The Strain-gauge plethysmography (SGP) was used to assess RAS and local capillary filtration was studied with the VSC (applied on the perimalleolar region); the disappearance of the weal was measured (minutes). The authors reported significant improvement with 34% reduction in rate of ankle swelling as compared with before treatment and reduced the Vacuum Suction Chamber time by 48%; with $p<0.02$. The authors concluded that TTFCA treatment in chronic venous insufficiency could start as early as development of leg edema to control deterioration leading to leg ulcerations. Authors suggested the complex actions on the microcirculation was essential for reducing edema [147].

Chong KJ et al, 2013, conducted a systematic literature search to evaluate the effect of *Centella asiatica* for improvement of symptoms and signs of chronic venous insufficiency (CVI) from 13 large electronic databases including the Cochrane Central Register of Controlled Trials for randomised controlled trials. The risks of bias were reviewed independently by two authors for studies selected. There were 8 studies showed that *Centella asiatica* significantly improved microcirculatory parameters such as transcutaneous partial pressure of CO_2 and O_2, rate of ankle swelling and venoarteriolar response. Three out of the eight studies did not provide

quantitative data. However, these studies reported that patients treated with *Centella asiatica* significant improved CVI clinical signs and symptoms such as pain, oedema and leg heaviness. These authors concluded that *Centella asiatica* may be beneficial for improving signs and symptoms of CVI but suggested further studies for adequate interpretation with caution since most of the studies contained missing data [148].

Enck, Paul et al, 2011 [149**]** suggested that there was a certain degree of placebo and drugs response in clinical trial which could range from 32**%** to 52**%** depending on the type of designed and both **Kirsch, I and LJ Miller, 1988 [**150**]** suggested that event though the double-blinded approach to abolish bias in placebo-response, the way administration of a placebo by elaboration to the participants could emerge with deceptive placebo response.

Miller FG. Wendell D and Swartzman LC, 2005 [151**]** had also suggested that proposed trial design by the so-called "balanced placebo design" **(**BPD**)** to separate the true drug effect and the true placebo effect from compound effect in clinical trials though two major disadvantages exist such as **(**1**)** it could provide false or correct information before participants could have been assigned the test drugs and **(**2**)** this information provided to participants before drug testing may raise suspicion among subjects as to which the information should have been provided, which ultimately could lead to deceptive placebo response. In other words, the information as well as the application of either medication and placebo could lead

to both true/false positive and true/false negative respectively.

Moreover, **Fischer JE, 2008 [**152**]** had also mentioned that in such clinical trial despite the placebo controlled trial, the placebo response exists, due possibly to perceived therapeutic efficacy of any given medication labelling package similar to commercial product, in this case the labeling of placebo with the same commercial feature similar to drugs itself could lead to deception of therapeutic response

CHAPTER III

METHODOLOGY

3.1 PREPARATION OF ACEMANNAN-ASIATICOSIDE TEST PRODUCT

The preparation of Acemannan-Asiaticoside test product employed standard preparation procedure in pharmaceutical formulation including (1) Acquisition of standard raw material of reliable standard herbal extract sources. (2) Preparation of gel-bases containing Acetylsalicylic Acid microencapsulation preformulation. (3) Preparation of finished herbal medicine combination as a test product containing Acemannan-Asiaticoside as active treatment and an identical control treatment without Acemannan-Asiaticoside. (4) Analysis of absorption employing Franz cell diffusion studies based on ASA microencapsulation (5) Microbial analysis of Acemannan-Asiaticoside test product and a control product. All preformulation, formulation and production process conducted at the pharmaceutical laboratories of the Eastern Asia University School of Pharmacy, Pathum Thani under the supervision of Asst Prof. Wichien Thanindratarn (Industrial pharmacy and Pharmaceutics Labs), Asso Prof. Dr. Nongluksana Sriubolmas (Microbiology Labs).

3.2 RESEARCH DESIGN

The research design was an interventional, prospective randomized, double-blinded, controlled trial. Participants were prospectively recruited and the interventions were randomly allocated product with concealment either active or control as double-blinded between participants, research nurse and physician by employing and a computer-generated two set of randomized numbers via consecutively randomization of two treatments.

3.2.1 Intervention

Participants fulfilling the eligibility criteria with inclusion-exclusion fulfillment were randomly blinding consecutively by allocation into one of two arms or one of two sets of concealed test product, either product 1 or product 2 as double-blinding as followed:

Each of blinding allocation remained concealed throughout the whole investigation as double-blinding between patients, physician and research nurses. In each set of products (either 1 or 2) contained labelling code of computerized randomization numbers on the tube of products in order to ensure the allocation of test products in a concealed package matched between each patients' case record forms and the concealment of test allocation, which secured labelling code in the same series of follow- visits. Randomization were consectively blinding during

allocation between researcher nurse whom supplied the concealed test products to patients whom also independently responded to outcome assessments for the diseases adapted with patient symptoms score and two sets of health-related quality of life questionnaires [34-38] and also co-ordinated for obtaining the diseases specific adapted symptoms for physician–rated symptoms persception score (PRSPS) [34,35] and the physisican whom comducted physical examination for assessment of venous severity clinical scores [32,33], all of whom had no prior knowledge of the allocated treatment.

3.2.1.1 Active treatment arm: each participant was given the herbal medicine combination gel formulation containing 2% asiaticoside plus 1% acemannan in a 2% ASA bases in bees wax microencapsulation. It was gently applied 10-15 ml on the leg with symptoms once in the morning and again at bedtime continuously for 3 months or 12 weeks. At each of the early weekly visit, patients shall be provided 2 tubes of the test products thereafter 8 tubes for each of the next four weeks of visit. At each of the follow-up visit, all patients were instructed to return the test products.

3.2.1.2. Control treatment arm: each participant was given only a bases preparation containing 2% ASA bases as controlled-gel with identical odor and color with no active herbal drugs. It was gently applied 10-15 ml on the leg with symptoms once in the morning and again at bedtime continuously for 3 months or 12 weeks. At

each of the early weekly visit, patients shall be provided 2 tubes of the test products thereafter 8 tubes for each of the next four weeks of visit. At each of the follow-up visit, all patients were instructed to return the test products.

3.3 STUDY POPULATION AND SAMPLE

The study population was Thai patients diagnosed as chronic venous disease based on CEAP Classification. The study sample was chonic venous disease patients diagnosed by surgean as CEAP class 1 to 4 and recruited form Somdet Prayanna Sangworn Chiang Rai and or Chiang Rai Hospital. These patients were ambulatory patients fulfilled the eligibility requirement and conformed inclusion and exclusion criteria. The patients were prospective consecutively recruited as per eligibility/inclusion and exclusion criteria, interviewed and screened where informed consent signed and obtained, diagnosed as CVD and classed either CEAP 1 to 4 as by surgeon. They were randomized to receive concealed treatment allocation as doubled-blinded. The treatments were either an Active Treatment Arm (Treatment 1) (The herbal medicine combination gel formulation containing 2% Asiaticoside plus 1% Acemannan in a 2% ASA bases in bees wax microencapsulation) or a Control Treatment Arm (Treatment 2) (the bases preparation of 2% ASA bases in bees wax microencapsulation as controlled-gel with identical odor and color. Patients were instructed to gently applied each 10-15 ml to the leg with symptoms twice in the morning and at bedtime continuously for the period of treatment duration of 3

month or 12 weeks. Both treatments were provided in sealed aluminum tube with similar external appearance labeled as either treatment 1 or treatment 2, with a given code well-stored at 20-25°Celsius by researcher nurses prior to dispensing. The researcher nurses were responsible for dispensing treatment, either treatment 1 or treatment 2 as per designated randomization number. The randomization was a 1:1 concurrently assigned to patients as consecutively. Patients were followed up every week up to 4 weeks then every 4 weeks up to 12 weeks of treatment. There were 6 visits (excluding the first day of randomization) subsequence to Day 1 as baseline assessment after which randomization and appointment for (1) visit-1 or 1 week, after the initiation of randomization. Later, patient was appointed for the 2nd, the 3rd, the 4th visits at Day 14 (week 2) visit-2, Day 21 (week 3) visit-3 and Day 28 (week 4) visit-4. Then patients were appointed for another additional 2 visits after 4-8 weeks as Day 56 (week 8) visit-5 and Day 84 (week 12) visit-6 as the end of the trial respectively. In each of follow-up visits, necessary information required as given in the clinical record form were obtained directly through patient interviews. Researcher nurse assessed (PRSP) whereas surgean clinically examined for VCSS. Two medical outcomes study as health-related quality of life scores, MOS SF-12 and MOS CIVIQ 14 interviews were obtained from patient assisted by researcher nurses at baseline at initiation before randomization (Day 1), after the end of the investigation (week 12). For patients' Self-Rated Symptoms Score as visual analog scale (VAS) (PSSS) were obtained weekly

symptoms perception rated by patients at all 6 follow-up visits respectively. The dairy report of adverse drug reaction and/or events (ADR/ADE) was a spontaneous patient report in the patient diary, whereas any added treatments or necessary test was collected at the time of each visit as given in clinical record form provided. The trial initiation and follow-up assessment during 12 weeks was given overleaf.

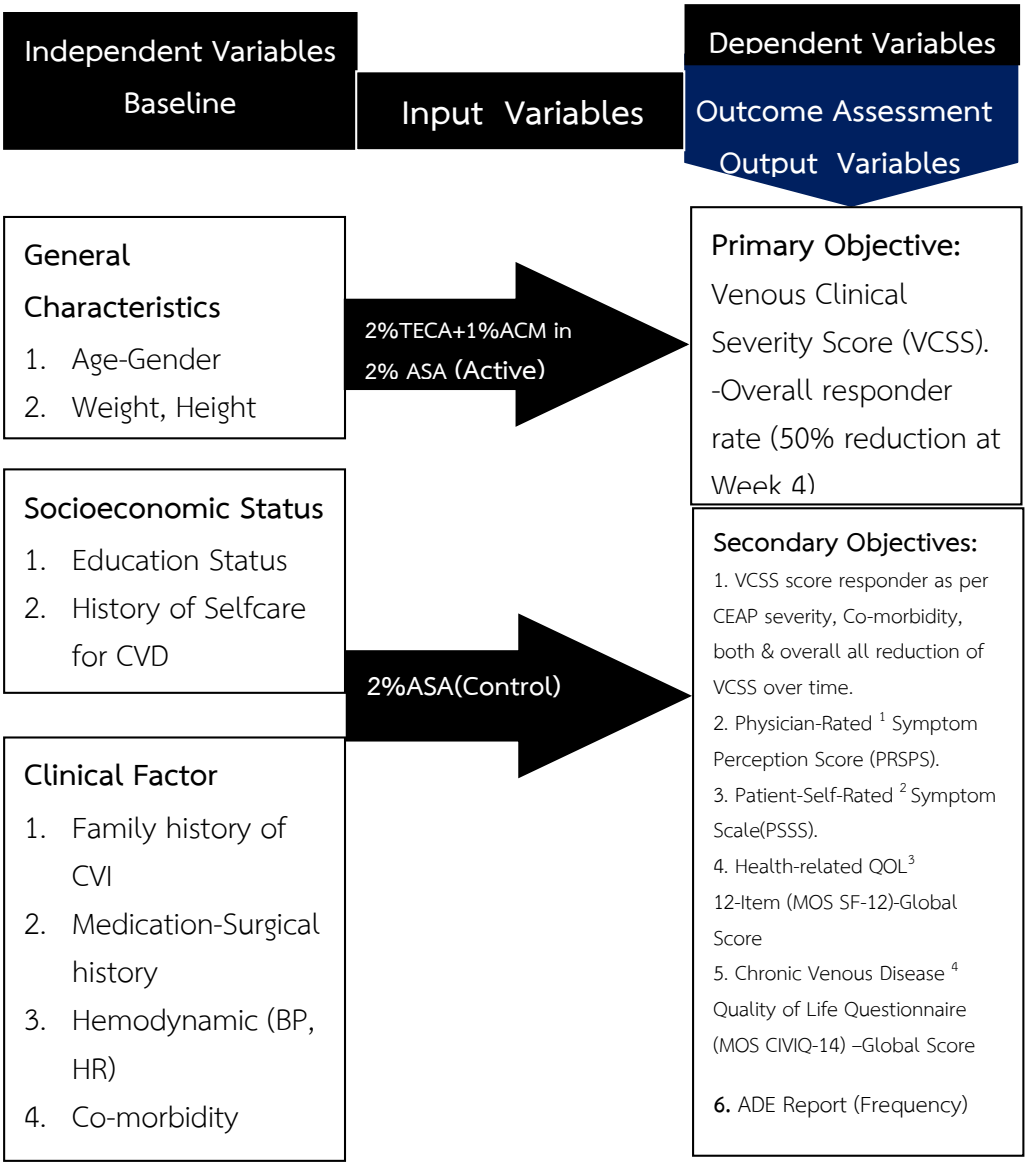


Diagram 1. Conceptual Framework

Note. CEAP = Comprehensive Classification System for Chronic Venous Disorders which CEAP classification include a description of the clinical class (C) based upon objective signs, the etiology (E), the anatomical (A) distribution of reflux and obstruction in the superficial, deep and perforating veins, and the underlying pathophysiology (P), whether due to reflux or obstruction [31. *Duplex Diagnostics employed for differentiation in selected cases. [1, 2, 3, 4] Validated Thai Version; # Mean weekly ADR reported by patients

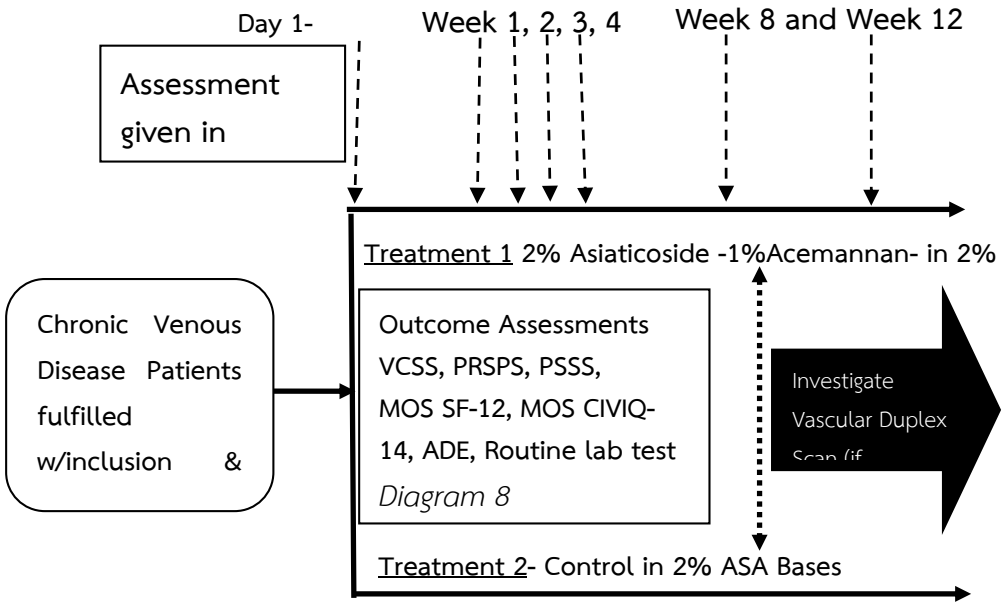


Diagram 8. Schedule of Assessments and Visits

3.3.1 Population of patients with leg symptoms due to CVD diagnosed by physician with CEAP Class 0-4.

3.3.2 Sample of patients with leg symptoms due to CVD diagnosed by surgeon with CEAP Class 0-4 at Wiang Chai Somdej Prayanna Sangworn Hospital/Chiang Rai Hospital

3.4 SAMPLE SIZE DETERMINATION

3.4 SAMPLE SIZE DETERMINATION

3.4.1 Sample size calculation [149-151,153,154]

The estimate response to active treatment based on 50**%** reduction of objective score of Venous Clinical Severity Score **(**VCSS**)** evelauted by physician as compared with a control treatment will be account for response to treatment**.** As such, sample

size shall be computed based on responder rate (%) for 2 independent proportions. The sample size estimation will be based on primary objective, improvement or responder rate as compared active treatment with a controlled treatment (Control) as two independent proportions after 4 weeks of treatment. Responder patients will be assessed with at least 50 % score reduction from baseline score after four weeks of treatment. As such, the sample size estimation based on one sample test of 2 independent proportions to show superiority of responder to treatments. Based on the hypothesis for normally distributed of probability of responder for test of difference in 2 independent proportions from which ¶1 and ¶2 are responders of active treatment arm (¶1)and control treatment arm and (¶2) the primary efficacy endpoint is responder rate after 4 weeks of treatment with following hypothesis:

Null Hypothesis H_0 : ¶0 - ¶2 = 0

Alternative Hypothersis H_1 : ¶1 - ¶2 ≠ 0 (2-sided test), alpha = 0.05,

At 80% power to detect the different, (ß) Beta =0.20)

¶1= True (population) response rate in group 1 (active treatment)

¶2= True (population) response rate in group 2 (control treatment)

Based on a balanced controlled designed, especially with the deception in research on the placebo and drugs effect, suggesting a possible placebo and drugs response could be found from 32% up to 52% (E, Paul et al, 2011; I, Kirsch et al, 1988; FG, Miller et al, 2005) [151-153]. As such with P2, assumed responder rate of Control Treatment Arm at 40% in the middle range for a placebo response rate or P2= 0.4 and P1, responder rate of Active Treatment Arm will assume 35% more responder

than Active Treatment Arm at 40**%** **+** 35**%** **=** 75**%** or P1**=** 0.75 whereby P**=** **(**P1**+**P2**)/**2, and Q1**=**1**-**P1, Q2**=**1**-**P2, and Q**=**1**-**P**.** As such the formular for calculating the significant different based on the Chi**-**Square test is as following given below**:**

$$n/group = \left[\frac{Z_{\alpha/2}\sqrt{2pq} + Z_{\beta}\sqrt{p1q1 + p2q2}}{P1 - P2}\right]^2$$

Without continuity correction

P1**=**0.40, Q1**=**0.6

P2**=**0.75, Q2**=**0.25

P **=** **(**P1**+**P2**)/**2 **=** **(**0.40**+**0.75**)/**2 **=** 0.575, Q **=** 1**-**P **=** 1**-**0.575 **=** 0.425

At Alpha **=** 0.05, $Z_{0.05/2} = Z_{0.025}$ **=** 1.96 and Beta **(**ß**)** **=** 0.2 0r 80**%** Power, $Z_{0.2}$ **=** 0.842

Sample Size $n_1 = n_2$ **=** N was calculated based on formula above as given**.**

N/group=$[[1.96\sqrt{2X(0.575)(0.425)}+0.842\ \sqrt{(0.40)(0.60)+(0.75)(0.25)}]/(0.75)-(0.4)]^2$

$= [[1.96\sqrt{2X\ 0.2443} + 0.842\ \sqrt{(0.24)+(0.18)}]/(0.35)]^2$

$= [[\ 1.96\ \sqrt{0.4886} + 0.842\ \sqrt{(0.4200)}]/(0.35)]^2$

$= [[\ 1.96\ X\ 0.6989 + 0.842\ X0.6480]/(0.35)]^2$

$= [[1.3698 + 0.5456]/(0.35)]^2$

$= [[1.9154]/(0.35)]^2$

$= [5.472]^2$

$= [29.94]$ **, n/goup** **=** **30**

The total sample size could be 30 each group with 10**%** drop outs or missing of 6 patients, therefore total sample size should be 66 patients**.**

3.4.2 Sampling Procedures

Eligible participants with leg symptoms complaints seeking advice at ambulatory surgery clinic were informed about the study by two researcher nurses. The paticipants recruitment started since 1 November 2015, for the period of 3 months as anticipated until numbers of eligible patients needed estimated for 66 patients was obtained. Once participants agreed to participate, they were explained in details of the study, independently interviewed then explained study details before referring participants to surgean for physical examination, diagnosis, CEAP grading of CVD before inclusion in the study. Only CVD patients diagnosed and graded were included in the study. Patients included in the study counter-signed informed consent, refered to conceal treatment allocation and appointment for follow-up by two researcher nurses. Patients sampling conducted as consecutively random sampling and concealed treatment allocations were blinding randomization to receive Treatment 1 or 2.

3.4.3 Participant Eligibility Criteria/Inclusion Criteria

Patients seeking advice at the surgery ambulatory clinics with at least two of the following symptoms:

1. Heavy leg
2. Pain in the leg
3. Sensation of leg swelling
4. Night cramp

5. Itching legs – or Pin in the leg

6. Sensation of burning

7. Sensation of severe pins/needle in the leg

8. Presence of superficial varicose vein

9. Presence of spider veins

10. Presence of blood-clotting.

And must have been accurately diagnosed as CVD by surgeon as per WHO- ICD10 Criteria and being graded as appropriate staging of the chronic venous insufficiency staging as per the CEAP Criteria.

3.4.4 Participants Non-inclusion/Exclusion Criteria

Patients with known history of:

1. ASA-exacerbated respiratory disease (AERD) and/or asthma

2. ASA-sensitivity and Rhinitis/Nasal Polyps

3. ASA and NSAIDs induce cutaneous reactions with Urticaria, Angioedema

4. ASA-induced anaphylactoid reactions with Hypotension, swelling, laryngeal edema, generalized pruritus, tachypnea.

5. Patients with known sensitivity or allergic to Titrated Extracts of Centenella Asiatica (TECA) or Asiaticoside.

6. Patients with known sensitivity or allergic to acemannan and the aloe vera products.

3.4.5 Outcome measures (Dependent Outcomes)

3.4.5.1 Primary Outcomes

Responder rate (%) looking at venous disease severity, assessed by the venous clinical severity score (VCSS) 50% score reduction measured at week 4 after baseline in general.

3.4.5.2 Secondary outcome measures

1. Responder rate (%) looking at venous disease severity, assed by the venous clinical severity score (VCSS) 50% score reduction measured at 4 weeks after baseline as per CEAP class, co-morbidity and combination

Disability caused by venous disease as other 2. venous clinical severities assessed by VCSS after Week 4,8 and at the end treatment (Week 12) [34,35]; 3. assessed by the Physician-Rated Symptom Perception Score (PRSPS); 4. Patient Self-Rated Symptoms Score (PSSS) measured at baseline, week 1,2,3,4,8 and 12; 5. Health-related Quality of Life Score Assessed with a Short-form 12-item Health Related Questionnaire Self-Administered Survey (MOS SF-12); 6. Health-related Quality of life, assessed by the Medical Outcomes Study Chronic Venous Insufficiency Quality of Life Scale International Collaboration 14 questionnaires (MOS CIVIQ-14) measured at baseline and week 12; 7. Adverse event incidental (as frequency) reported by patients in a weekly diary follow-up.

3.5 STUDY PROTOCOL AND RESEARCH TOOLS

The study protocol was registered at the international clinical trial registry with the registration trial number # ISRCTN54360155 DOI 10.1186/ISRCTN54360155 available at http://www.isrctn.com/ISRCTN54360155 with over all description of research tools

and other detail**.** The case report form as part of research tools for data transfer are provided in the appendix**.** Details of research tools other than the Duplex Scan **(**if need for specific confirmation by surgeaon is available at the hospital**).** General tools were as following**.**

3.5.1 VCSS, the **Venous Clinical Severity Score** as suggested by Rutherford et al [32] and later confirmed for its specificity by Meissner et al [33] are objective assessment questionnaires for specialist to evaluate the seven domains of CVD symptoms and characteristics. These symptoms included (1) pain, (2) varicose veins, (3) venous edema, (4) skin pigmentation, (5) inflammation (6) induration (7) presence of active ulcers including numbers of ulcers and duration of ulcerations. The score ranges from 0 (absence) to severe score (3). Each of the item of venous clinical severity score must be converted into percentage score representing overall severity from 0 to 100, as such the final score computation employed a transformed percentage scores from absent (0) to severe (100). In each items of the score were transformed into percentage score by multiplication of 25 percentage points to each of raw score eg. Raw score 0, then percentage score was 25X0 = 0 whereas then Raw score =1, 2 or 3 then percentage score was 25X1 = 25 points, 25X2 =50 points and 25X3=75 points respectively. Overall VCSS was the summation of the overall clinical severity domain as percentage score [31,32].

3.5.2 PRSPS, the **Physician-Rated Symptom Perception Score** as suggested by Talley et al [34] was adapted to CVD which was an objective assessment questionnaire of disease specific symptoms for specialist to evaluate the selected all of any relevance clinical symptoms included (1) Heavy leg (2) Pain in the leg (3) Sensation of leg itching (4) Night cramp (5) Itching leg (6) Sensation of burning (7) Sensation of Oin/Needle in the leg (8) Superficial varicose veins (9) Presence of spider veins. The PRSPS was a modified adapted with disease specific symptoms adjustment for chronic venous disease. The score ranged from 1(none) to most severe score (5). The PRSPS score assessed as symptoms severity as such the final score computation employed a transformed percentage score ranging from absent (0) to severe (100). In each items of the score were transformed into percentage score by multiplication of 20 percentage points to each of raw score eg. Raw score 1, then percentage score was 20X1 = 20 whereas then Raw score =2,3,4 or 5 then percentage score was 20X2 = 40 points, 20X3 =60 points, 20X4=80 points and 20X5 = 100 points respectively. Overall PRSPS score was the summation of the overall sympoms severity score for all reported symptoms as percentage score [34,35].

3.5.3 PSSS, the **Patients-Self-Rated Symptom Scale** is a visual analog scale from 1(none) to 10(the most severe). It was a subjective self-assessment of all selected most relevant patients perceived CVD symptoms included (1) Pain (2) Heavy leg (3) Itching in the leg (4) Needle or Pin in the leg (5) Night cramp (6) Leg edema and (7) Any other resported symptoms. These were measured in centrimeters rated by

patients as suggested by Talley et al [35] with a modified selected CVD symptoms. The final score computation employed a percentage scores from 0 to 100 for interpretation. The PSSS score assessed as symptoms severity as such the final score computation employed a transformed percentage score ranging from absent (0) to severe (100). In each items of the score were transformed into percentage score by multiplication of 10 percentage points to each of raw scales eg. Raw scales 1, then percentage score was 10X1 = 10 whereas then Raw score =2,3,4 and to 10 then percentage score was 10X2 = 20 points, 10X3 =30 points, 10X4=40 points and to 10X10 = 100 points respectively. Overall PSSS score was the summation of the overall sympoms severity score for all presented symptoms as percentage score [34,35]

3.5.4 MOS SF-12, was a self-rated health survey by patients for objective self-assessment of health-related quality of life, known as the 12-item Short Form Medical Outcomes Study or in short known as **SF-12** proposed by Ware JE et al [38]. In this investigation, The MOS SF-12 Thai version, as a validated translation was employed. The MOS SF-12 scoring was employed for MOS SF-12 similar to SF 12 for physical function (PF). role limitations because of physical health (role-physical, RP), bodily pain (BP), general health perceptions (GH), vitality (VT), social functioning (SF), role limitations due to emotional problems (role-emotional, RE), and mental health (MH). The scoring domains divided into Physical Component Summary score (PCS)

(Items 1,2,3,4,5 and 8), Mental Component Summary score (MCS) (items 6,7,9,10,11 and 12) and global score (all items). The computation of score were computed as assigned percentage scoring from each item response yes/no (1/0) and combined with a scoring from a categorical response choice (1 to 3, or 1 to 5), the same for reverted questionnaires where the score was reverted accordingly. These were then converted to assigned percentage score between 0 to 100 on each items, each domain. The higher scores reflected a better health-related quality of life and vice vesa for the lower score. The MOS SF-12 percentage score were employed for comparsion between the active and control treatment as well as comparison effects of treatment from baseline (day 0) to the end of trial (week 12).

3.5.5 MOS CIVIQ-14, was structured specific health survey questionnaires for chronic venous disease patients by patients for assessment of health-related quality of life with 5-point Likert scale to rate the importance of leg problem, originally was a 20-items and had been further re-assessed with original adaptation to a 14-item which measured physical, psychological and social functioning and pain known as the CIVIQ-14, an international disease specific hrealth-related quality of life score [36,37]. There were 14 items which divided into (1) Pain Score (item1), (2) Physical Function Score (items 2,3,4,5,6,7 and 8), (3) Psychological and Social Function Score (items 9,10,11,12,13 and 14). There are 14 questions in the CIVIQ-14, each with 5 possible answers (1 to 5), the minimum possible score being 14 and the maximum 70. In

order to calculate the Global Index Scoe (GIS), the difference between the final score and the minimum possible score was divided by the difference between the theoretical maximum and minimum scores (70-14=56), multiplied by 100. GIS = ([Final score – minimal possible score] / [Theoretical maximal – minimal score]) x 100. The final responding physical function, psychological and social function score were assigned with a percentage point corresponding with each items used in the scoring similar to global index scoring as suggested by authors. For, this investigation, the computation of MOS CIVIQ 14 were reflected as Pain Score, Physical Function Score., Psychological and Social Function Score and Global MOS CIVIQ-14 score. The score comparison reflected both the active and control treatment arms from baseline (day 0) and at the end of the trial (week 12) as well as treatment from baseline to week 12. This investigation emnployed the Thai version of validated translation CIVIQ 14.

3.5.6 ADE was the adverse drug event self-reported incidence by patients. Since the treatment was a topical administration where mostl likely skin reaction which had been problematics and reported in an early pilot trial in healthy volunteer, the study suggested the incidence though was likely due to the active durg but pharmaceutical adjuvant in the formulation may caused the same and mainly external skin reaction. Such that, ADE data reported in the study intended mainly frequency of incidence occurred not the objective of finding out the specific adverse events report [160].

The ADE reported in this investigation intended a structure set of responding questionnaires reported as presence or absence as (1) tolerate adverse drug event, (2) no adverse drug events (3) serious adverse drug evemt leading to stop administration. The frequency of ADE report was a weekly summarized in patient calendar at follow-up.

3.6 DATA COLLECTION

Two researcher nurses were contracted and trained to be familiar with the clinical trial protocol in terms of patient interviews, assessment tools required by the trials and the provisional of interventional drugs for trial during randomization. Moreover, researcher nurses played vital role in overall randomized double-blind procedures where both patient and investigator and research blinded to the allocation intervention. The treatment allocation concealment kept blinding throughout the trial. The final analysis subsequence to data collection performed independently by researcher and the code revealed at later stage. Data collections with respect to patient personal history, socioeconomic information were completed in the case record form at the time of recruitment during trial initiation. Other medical and clinical data were completed by investigator and researcher nurses with corresponding to each patient. All data and information was transferred into data collection, clinical record forms for further data management. Data monitoring with respect to correct records and audit was performed periodically by researcher at the

site of study. Researcher was centralized and available to all provision supports and should any doubts of the research procedure to ensure the smooth running of the investigation.

3.7 DATA ANALYSIS

This study employed SPSS version 17 for data management and analysis

3.7.1) Descriptive statistic such as frequency in percentage, mean, standard deviation, minimum, maximum was applied for the demographic characteristics including age- education status, marital and socioeconomic status and clinical characteristics including medical-surgical history, medication history and leg symptoms including concurrent disease and CEAP diagnosis classification of CVD. The baseline comparisons between group treatment between controlled treatment and active treatment was performed.

3.7.2) Statistical analysis for descriptive statistics will be employed with the hypothesis testing based on assumption that active treatment should provide 35% more responder rate than standard treatment, whereby responder rate was defined by the standardized Venous Clinical Severity Score (VCSS) as a transformed percentage score in terms of 50% score reduction from baseline after 4 weeks of treatment defined as responder. In this regards, the assumed standard treatment responder rate is allowed as high as 40% and as such the active responder rate

should be as high as 75% responder rate. The overall statistical analysis was performed for primary and secondary outcomes with **input variable as nominal (treatment 1 or 2)** for various independent variables and **output variable** as outcomes or dependent variables (ratio, quantitative discrete or quantitative normal) as follows:

1. **Chi-square test** (χ^2-test) for responder rate with the minimum 50% reduction of score from baseline as responder rate after 4 weeks of treatment for VCSS score between active treatment (Tx 1) **VS** controlled treatment (Tx 2) as primary outcomes for categorical outcomes (ratio) for one sample two independent group.
2. **T-test Analysis** (assumed normal distribution) for comparison of mean score differences within treatment (Tx 1 or Tx 2) at baseline – Week 4, 8 and 12 for **VCSS** total score as secondary outcomes.
3. **T-test Analysis** comparison of both global score, physical component summary score (PCS) and mental component summary score (MCS) mean score difference between active treatment (Tx 1) **VS** controlled treatment (Tx 2) for global score of **MOS SF-12, MOS CIVIQ -14** between baseline score **(**Day 0**)** and at the end of the trial **(**Day 84, or week 12**)** as secondary outcomes**.**

4. **Paired T-test and T-test** Analysis performed for each of scorenand domain score of MOS CIVIQ 14 and MOS SF 12 comparison before and after each of treatment, and comparison between active treatment (Tx1) and control treatment (Tx 2)
5. **Fisher Exact Test** for total spontaneous adverse events report comparing between two groups with non-parametric statistics as secondary outcomes **(**quantitative discrete**).**
6. The analysis shall assume 80**%** Power effects and with significance level at $p<0.05$.

3.7.3 Summary of Statistical Analysis

Independent variable or Input variable	**Dependent variable or Outcome variable**	**Type of Data**	**Statistics Used**
1. Demographic characteristics 2. Clinical Characteristics		Continuous & Categorical data	Describing in frequency as ratio (%, Mean, Standard deviation, Range, Min-Max),

			Descriptive Statistics (**T-test** for continuous data and **Chi-Square Test** for categorical data)
TX 1 (Active) VS TX 2 (Controlled)	Venous Clinical Severity Score (VCSS) evolution from baseline to Week 4,8 and after 12 weeks.	Continuous data	Score describing in frequency (Mean, Standard deviation, Range, Min-Max), **T-Test Analysis**
TX 1 (Active) VS TX 2 (Controlled)	Responder rate (Score reduction 50% from	Cateogircal data	**Chi-square test** (χ^2-

	baseline after 4 week)		test)
TX 1 (Active) VS TX 2 (Controlled)	Score between each treatment arms for comparison of mean score differences **b/w TX 1 VS TX 2 from** baseline to W-4, 8 & W-12	Continuous data	T-Test assuming normal distribution
TX 1 (Active) VS TX 2 (Controlled)	**PRSPS, PRSS** Score from Baseline to W 4,8,12. And **MOS SF12, MOS CIVIQ14 score -** baseline to W-12	**Continuous data (Rated Score)**	Score describing in frequency (Mean, Standard deviation, , Range, Min-Max)
TX 1 (Active) VS TX 2 (Controlled)	**Score before and after** each treatment arms for comparison of mean score differences b/w	**Continuous data (Rated score)** for **PRSPS, PRSS,**	**T-test Analysis (assuming normal**

	baseline W-4, 8 & W-12	**MOS SF-12, MOS CIVIQ-14**.	**distribution**)
TX 1(Active) VS TX 2 (Controlled)	Total number of spontaneous adverse events report comparing b**/**w two groups at the end of the trial **(**W 12**)**	Quantitative discrete	**Fisher's Exact Test**

3.7.3) Analysis of results and handling of missing data (156-157)

3.7.3.1) Analysis of the results shall be performed based on **per protocol analysis** which shall include all recruited patients based on protocol. The analysis will ensure that patients in the trial have balance effects of exposure to treatment of investigating drug in their own context of their socioeconomic and drug consumption behaviour. The analysis could be consistent though presenting shortcoming of practical implication and lack of wider generalizability as nature of controlled trials.

3.7.3.2) Handle of missing data (155-157)

Practically, preventing of missing data shall be required to ensure power of the trial as well as reduction of data variability due to insufficient sample size**.** The additional recruitment of presumed 10**%** dropouts shall be employed to ensure sufficient sample size**.** However, in certain circumstance, missing data should inevitably appear which could be determined as follows**:**

1. Missing Completely at Random (MCAR) This type of missing data defined with the mechanisms as the **dependent variable** with the probability of an observation being missing **does not depend on observed or unobserved measurements at random, such as drop outs**.

2. Missing at Random (MAR) This type of missing data defined with the mechanisms as the **dependent variable** with the probability of an observation being missing depends on observed measurements at random, such that behavior of the post drop outs observations could be anticipated from the observed variables such as **dropout which may be due to lack of efficacy or poor efficacy.**

3. Missing Not at Random (MNAR) This type of missing is **neither MCAR nor MAR** is the probability of an observation being missing depends on unobserved measurement**.** As such, value of the unobserved responses which depends on information not available for the analysis and therefore future observations cannot predict without bias from model**. Patient continues visits with good results**

expected but suddenly drop outs due to lack of efficacy, as such it is irrational to anticipate patients to assume benefits from treatment that based on observation data that may be likely to predict good outcomes.

4. Report of Missing Data

The report for difference between treatment groups in the proportion of patient withdrawals. The report for difference between treatment groups with timing of withdrawal and reason for patient withdrawals.

There is no missing data but encountered only insufficient recruitment as planned in dued time and as such this investigation was intended to analysis as per protocol analysis.

3.7.3.3) Analysis of Missing Data (155-157**)**

1. Single censure to replace a missing data point by a single value and analyses are conducted as if all the data were observed.

2. In case of situations where data collection is interrupted before the predetermined last evaluation time-point, the imputation method shall employ the Last Observation Carried Forward **(**LOCF**)** meaning the last measured value of the endpoint to all subsequent, scheduled, but missing, evaluations shall be assumed with assumption that LOCF produce an unbiased estimate of the treatment effect.

3. In certain circumstance, LOCF does not produce conservative estimates such that patient's condition is expected to deteriorate over time, the LOCF will produce exaggerated optimistic results for both treatment groups, and if the withdrawals on

the active group are earlier (e.g. because of adverse events) the treatment comparison will certainly provide an inappropriate estimate of the treatment effect and may be biased in favor of the test product. Hence in this situation an LOCF analysis is not considered appropriate.

4. Baseline Observation Carried Forward (BOCF) is a single censure approach that may be appropriate where and when a patient withdraws from treatment it may be reasonable to assume that effects such as pain return to its baseline level and that the patient does not, in the long-term, derive benefit from treatment.

5. Other simple approaches for single censure of missing data are to replace the unobserved measurements by values derived from other sources. Possible sources include information from the same subject collected before withdrawal, from other subjects with similar baseline characteristics, a predicted value from an empirically developed model or historical data. (e.g. assigning the worst possible value of the outcome to dropouts for a negative reason (treatment failure) and the best possible value to positive dropouts (cures)). Regression methods for sensitivity analyses

6. To employ a different pre-specified censure data for each different reason for withdrawal. This technique provides flexibility in handling various reasons for and timings of withdrawal and their consequently possible relationship between missing data and the outcome of interest. Since unobserved sample data were mainly due to lost to follow-up at an early of the investigation during the pre-enrollment, as

such censure method was employed for this investigation for unobserved sample at ramdoms as loss-to follow up.

7. Certain statistical approaches to handling missing data which do not employ explicit attrition shall be explored for further application depending on type of data such as a mixed-effect model for repeated measures (MMRM)) for continuous data. The generalized estimating equations (GEE)) and random-effects (e.g. generalized linear mixed models (GLMM)) approaches for categorical data. The Likelihood-based methods (MMRM and GLMM) and some extended GEE (i.e. weighted GEE) models for MCAR and MAR assumptions.

3.8 ETHICAL CONSIDERATION

Under the guidance of College of Public Health Sciences, and hospital ethical authorities, all participants received both written and verbal information before they agreed to participate in the study. The patient informed consent was obtained from patients before participation to the study. The participants had their right to refuse to participate in the study and freedom to withdrawal at any time during the trial period. Their patient personal information was kept confidentially, and was not be used for other purpose.

CHAPTER IV

RESULTS

4.1 PREPARATION OF ACEMANNAN-ASIATICOSIDE TEST PRODUCT

4.1.1 Herbal Extracts Source

The preparation of Acemannan-Asiaticoside test product employed standard preparation procedure in laboratories for pharmaceutical formulation including the acquisition of herbal extract substance, Asiaticoside 40% and Acemannan 17%. As the two herbal extracts employed for the formulation of the herbal medicine combination test product were obtained from two global supplied international commercial manufacturing source of Asiaticoside, which was obtained as a Freezed-Dry 40% Asaiticoside from China. The extract was fresh prepared and well kept within 6 months after production at the time of pre-formulation preparation. Acemannan, was acquired by direct purchased form USA commercial source as 17% Freezed Dry Acemannan. The standards and analysis of the two herbal extracts were given in **the Appendix 1.**

4.1.2 Preparation of Acetylsalicylic Acid Encapsulation Preformulation (as control)

The preparation of Acetylsalicylic Encapsulation was carried out at the pharmaceutical laboratories of the Eastern Asia University School of Pharmacy. The

pre-formulation test process was performed before assembling into the large scale production and thereafter completed within a aluminium tube as an air-tight light-protcted packaging material as finished products. The former was a collaboration endeavor by faculty member and qualified laboratories technician from the same labs. In the process of pre-formualtion, other needed ingredients and adjuvants as in the formulation were obtained as standard substance, pharmaceutical grade and well prepared via standard production procedures, with self-contained analysis and quality control by the pharmaceutical laboratories of the Eastern Asia University. Sample photo of euipments, ingredients and processing as given in **Appendix 2.** The preparation procedures included:

(1) Preparation of Acetylsalicylic Acid in Beeswax microencapsulation by warmig both Acetylsalicylic Acid (Aspririn) and Beeswax upto 75 celcius on heater, then mixing both Asprin and Beeswax were mixed together and stir to form a liquefied aspirin in beeswax by keeping in 75 celcius. Diiferent concentration was calculated for true encapsulation, then pre-formulation of Microencapsulation test for released of Aspirin by Franz Cell performed to ensure selction of the best formulation to release the Aspirin. The Aspirin and UV Visible Curve and Franz Cell Test for Aspirin Release Test in **Appendix 3** and **Appendix 4.**

(2) Preparation of gel-bases containing preparation of Carbophol 940, Triethnolamine, Glycerine, Disodium-EDTA to form a gel-like structure base and Dimethy Isosorbide Dinitrate (DMN).

(3) Adding the (1) into (2) to form a gel-base standard preparation of Acetylsalicylic Acid in Beeswax micro-encapsulation. Adding solution containing Camphor, Tri-ethanolamine, Glycerine and Isopropyl Alcohol, Methy/Propyl Paraben solution in propylene glycol as early prepared into the final gel-like preparation. The standard Acetylsalicylic Acid in Beeswax micro-encapsulation, without the Acemannan-Asiaticoside Extract combination was employed as the controlled gel-base. Adjusting the gel-base with Dimethyl Isosorbide Dinitrate (DMN) for stabilization of aspirin encapsulation and to stabilize the gel-formation thereby preserved the stability for the product before packing and performed the **microbial test (Appendix 5)** and physical statbility test.

4.1.3 Preparation of Finished Herbal Medicine Combination

The preparation of Acemannan**-**Asiaticoside Extract Combination in Acetylsalicylic Acid Encapsulation Preformulation **(**as Herbal Extract Combination**)** performed by following**:**

(1) Preparation of Acemannan – Asiaticoside extract by dissolving the ingredients in the water, filtered for clarity and then mixing Triethonolamine and glycerine to form a formulation of Acemannan-Asiaticoside extract combination preparation.

(2) Adding the (1) in to the gel-base preparation of (4.1.2) to form the finish-product Acemannan-Asiaticoside Extract Combination in Aspirin-Beeswax micro-encapsulation. Adjusting the gel-base with Dimethyl Isosorbide Dinitrate (DMN) to stabilize the gel-formation and thereby preserved the stability for the product before packing and

performed the microbial test and physical statbility test. (Finished product filling in the 45 –ml Aluminium tupe for 35 ml and sealed as in a closed tight container **(Appendix 2).**

4.1.4 Physical stability test performed by incubating the gel-based product in the incubator at constant temperature of 45 and 75 celcius for 30 days.

4.1.5 Microbiological Analysis of Acemannan-Asiaticoside Test Product

The microbial test analysis of Acemannan-Asiaticoside test of herbal extract was performed as standard USP procedure by Associate Professor Dr. Nongluk Sriubolmad and the results was given **(Appendix 5).**

4.1.6 Absortion Analysis: Franz Cell Diffusion Studies

Acetyl Salicylic Acid (Aspirin) (%w/w)-Bee wax microencapsulation: Formulaiton Tested. Were as following**: P1** 1:4, **P2** 1.5:2.5, **P3** 2:3, **P4** 2.5:2.5, **P5** 3:2, **P6** 4:1 which then attempted by following procedure**:**

1. Preparation of UV maximum absorption curve using Phosphate Buffere at pH 7.4 as blank
2. Diisolved Aspirin at phosphate buffer ph 7.4 to test for UV maximum absortion curve**.**
3. Preparation of cellulose membrane **-** 20 mm immerged in normal sline 15 minutes followed by 10 mM Sodium Bicarbonate at 80 celcius for 30 minutes followed by immerged in 10 mM disodium EDTA for 30 minurtes**.** This cellulos membrane was kept in the ethanol 20**%** in refrigerator**.** When need to test this

membrane was immerged in the media solution for 30 minutes before testing.

4. The standard curve of Aspirin in phosphate buffer pH 7.4 – prepared by weighing 30 mg of Aspirin standard and dissolved in the test tube 15 ml – with phosphate buffer pH 7.4 - 5 ml as concentrated stock solution of 6 mg/ml then diluted to different concentration at 0.357, 0.75, 1.5, 3 and 6 mg/ml respectively Measure the UV visible spectrophotometry at 302 nm, it is expected that the absorbance would be in the range of 0.09375 to 3, form then the absorbance and concentration curve was constructed (absorbance as λmax) Y -axis and concentration of Aspirin in X-Axis as a linear regression equation.**(Appendix 3).**
5. At the time tested, gel-base preparation containing controlled (Aspirin) was placed for 5 gm on the the cellulos membrane and place on the Franz cell, sampling 50 ml of the solution from Franz cell at different time (0.1,2,3,4,5,6,8,9,10 and 24 hours) and measure against the concentration of Aspirin using UV visible spectrophotometry
6. The Acemannan-Asiaticoside Extract Combination of the Finished product was not tested as due to the standard of the combination remains controversial as such the test for Aspirin-Beeswax micro-encapsulation would sufficiently ensure the release of the active ingredients to passing though membrane. (The curve of Aspirin releaseing from gel-base was provided as below.
7. The Absortion Analysis: Employed the Franz Cell Diffusion Studies performed by - assuming Acetylsalicylic Acid as standard substance &UV-Visible

Spectrophotometer **(Appendix 6).**

8. All preformulation, formulation and production process conducted at the pharmaceutical laboratories of the Eastern Asia University School of Pharmacy, Pathum Thani under the supervision of Asst Prof. Wichien Thanindratarn (industrial Pharmacy and Pharmaceutics Lab.), Asso Prof. Dr. Nongluksana Sriubolmas (Microbiology Lab.).

4.2 BASELINE DATA CHARACTERISTICS AND TEST RESULTS

Subsequent to patient pre-recruitment initial screening at Chaing Rai, Wiang Chai Somdej Prayannasangwarn Hospital, only 120 patients were successfully pre-screening.

Among the pre-recruitment screening, there were 42 patients as planned for pre-enrollment from initial screening and therefore randomization with 42 patients as given in detail in **patient screening flow chart was provided in the Diagram 8 given overleaf.**

4.2.1 Patient Screening Flow-Chart

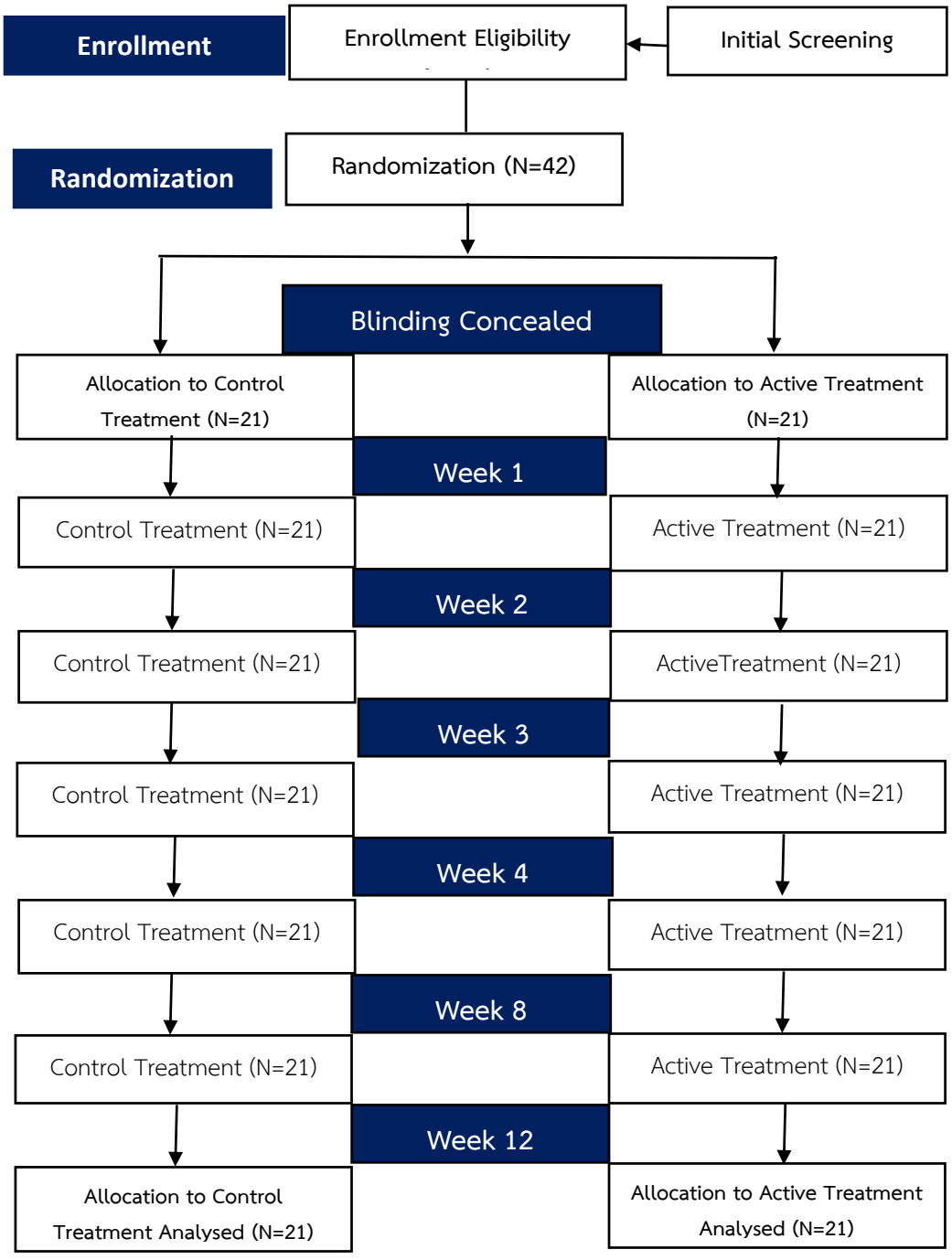


Diagram 9 Patient Screening Flow- Chart

Note. 1. Consecutive randomization **2.** Test products allocation were blinding b/w physician-nurse-patients **3.** Each of any one of two set of test products contained matching computerized allocated randomization numbers as series on products.

4.2.2 Demographic Characteristics of Patients

Among 42 patients, all were female gender, for both two treatments group. There was no significant demographic different among this two group, except a slightly different in marital status, details of which given in **Table 1.** overleaf:

Table 1 Demographic Characterisitcs

Demographic	Control (N=21) Mean (SD), Min-Max, Range	Active(N=21) Mean(SD), Min-Max, Range	P-Value
Gender Female	21	**21**	
Age (in years)	48.90 (6.09) 39-62, 23	47.00 (5.42) 39-58, 19	**0.291**
Weight (in Kg)	59.00 (6.05) 48-68, 20	60.81 (5.97) 49-74, 2	**0.336**
Height (in CM)	159.28 (6.93) 150-150, 2	160.23 (5.94) 149-170, 21	**0.636**
EducationStatus			
Graduate	4 (19.0%)	7 (33.3%),	
College	5 (23.8%),	11 (52.4%),	***0.157**
Secondary	12 (57.1%),	3 (14.3%)	
Marital Status			
Single	3 (14.3 %),	11 (50.0 %),	
Mariied	17 (80.9%)	10 (45.2%)	***0.025**
Divorced	1 (4.8%)	1 (4.8%)	

P-value by T-test, * P-value by Chi-Square Test

4.2.3 Clinical Characteristics of Patients.

All of 42 enrolled patients, applicable clinical characteristics were provided in Table 2 and Table 3 below and overleaf.

There was no significant different clinical characteristics for both the two treatments groups with the exception to slightly different for the numbers of presence of major complains on leg symptoms **(**p**=** 0**.**087**)** and except the minor isolated presence of Varicose Vein (**p**= **0.019**),** where patients in the control group slightly higher as compared with the active treatment group as given in **Table 2 and Table**. **3**. overleaf.

Table 2 Clinical Characterisitcs (A)

Clinical Characteristics	Control (N=21) Mean (SD), Min-Max, Range	Active (N=21) Mean (SD), Min-Max, Range	P-Value
Gender Female	21	21	
Family History of CVI	Yes (0), No (21)	Yes (0), No (21)	-
Medical His-Comorbid			
None	16 (76.2%),	17 (80.1%),	
Hypertension	5 (24.2%),	2 (9.7 %),	*0.191
Musculo-skeletal	0 (0%)	2 (9.7%)	
Co-Medic (Self-Care)			
Sometime	17 (82.5%),	16 (77.8%),	*0.707
Regular	4 (17.5%)	5 (22.3%)	
CEAP Classification			
CEAP C1	8 (38.0%),	10 (47.6%)	*0.533
CEAP C2	13 (62.0%)	11 (52.4%)	
SBP (mmHg)	138.95 (4.44) 129.0-146.0, 17.0	137.86 (4.06) 130.0-145.0,15.0	0.410
DBP (mmHg)	88.04 (3.90) 78.0-97.0, 19.0	88.57 (2.54) 83.0-92.0, 9.0	0.609
Heart Rate (tpm)	70.81(4.08) 64.0-78.0, 14.0	70.33 (4.68) 60.0-78.0, 18.0	0.727
Numbers of Leg Symptoms (symptom-Table 3)	3.71(0.902) 3 - 6, 3	3.05(0.669) 2 - 4, 2	*0.087

P-value by T-test, * P-value by Chi-Square Test

Table 3 Clinical Characterisitcs (B)

Leg Symptoms	Control (N=21) Number (%),	Active (N=21) Number (%),	P-Value
Heavy Leg			
Yes	7 (43.7%)	9 (56.3%)	*0.525
No	14 (53.8%)	12(46.2%)	
Leg Swelling			
Yes	6 (56.7%)	9 (43.3%)	*0.334
No	15 (40.0%)	12 (60.0%)	
Leg Paiin			
Yes	21 (50.0%)	21 (50.0%)	-
No	0 (0%)	0 (0%)	
Night Cramp			
Yes	12 (57.1%)	7 (33.3%)	*0.121
No	9 (42.9%)	14 (66.7%)	
Burning Sensation			
Yes	0 (0%)	0 (0%)	-
No	21 (100%)	21 (100%)	
Pin/Itching in Leg			
Yes	1 (4.8%)	0(0%)	*0.050
No	20 (95.2%)	21(100.0%)	
Leg Itching			
Yes	10(47.6%)	4(19.1%)	*0.311
No	11(52.4%)	17(80.9%)	
Varicose Vein			
Yes	18 (85.7%)	11 (52.4%)	*0.019
No	3 (14.3%)	10 (47.6%)	
Spider Veins			
Yes	3 (50.0%)	3 (50.0%)	-
No	18 (50.0%)	18 (50.0%)	

*** P-value by Chi-Square Test**

Results as per primary objective (Responder rate after week 4 and its sub-analysis)

4.2.4 Venous Clinical Severity Score (VCSS). Responder – Rate (Primary Objective)

The overall responder between the Active was better than the Control group at from week 4, 8 and 12 respectively as observed with p=0.003, p=0.009 and p=0.025 respectively given in table 4 below.

Table 4 Venous Clinical Severity Score (VCSS). Responder -50 % Score Reduction (all patients)

VCSS Total Score 50% Reduction	Control (N=21) Numbers with 50% Score reduction N/Total (%)	Active (N=21) Numbers with 50% Score reduction N/Total (%)	P-Value
After 4 weeks	2/21 (9.52%)	12/21 (57.14%)	*P=0.003
After 8 weeks	3/21 (14.28%)	12/21 (57.14%)	*P=0.009
After 12 weeks	4/21 (19.04%)	12/21 (57.14%)	*P=0.025

*P-value – Fisher's Exact

As a secondary objective, in addition, an analysis of the treatment effects or responder to treatment defined by a 50**%** reduction of the Venous Clinical Severity Score after week 4, founded that the responder for treatment by Active is better than the Control regardless of severity of disease Class **(**CEAP Class**) (**p**=**0.003**).** However, the CEAP Class 1 provided better responder to treatment **(**p**=**0.013**).** This better responder to Active treatments remain observed even after Week 8 **(**p**=**0.009**)** and Week 12 **(**p**=**0.025**)** as given in the **Table 5** overleaf

Table 5 Venous Clinical Severity Score (VCSS). Responder -50 % Score Reduction (Based on Severity CEAP Classification)

VCSS Total Score 50% Reduction	Control (N=21) Numbers with 50% Score reduction N/Total (%)	Active (N=21) Numbers with 50% Score reduction N/Total (%)	P-Value
After 4 weeks			
CEAP Class 1	0/8 (0%)	6/10 (60.0%)	*P=0.013
CEAP Class 2	2/13 (15.38 %)	6/11 (54.54%)	*P=0.082
All CEAP Class 1+2	2/21 (9.52%)	12/21 (57.14%)	*P=0.003
After 8 weeks			
CEAP Class 1	0/8 (9.52%)	6/10 (60.0%)	*P=0.013
CEAP Class 2	3/13 (23.07%)	6/11 (54.54%)	*P=0.206
All CEAP Class 1+2	3/21 (14.28%)	12/21 (57.14%)	*P=0.009
After 12 weeks			
CEAP Class 1	0/8 (9.52%)	6/10 (60.0%)	*P=0.013
CEAP Class 2	4/13 (30.76%)	6/11 (54.54%)	*P=0.408
All CEAP Class 1+2	4/21 (19.04%)	12/21 (57.14%)	*P=0.025
All responder after 12 weeks	4/21 (19.04%)	12/21 (57.14%)	*P=0.025

*P-value – Fisher's Exact

As a secondary objective, an analysis of the treatment effects or responder to treatment defined by a 50**%** reduction of the Venous Clinical Severity Score after week 4, founded that the responder for treatment by Active is better than the Control based on the presence of co-morbidity **(**Hypertension and Musculoskeletal Disorders**).** The Active group provided better responder in the patients with no co-morbidity **(**p**=**0.007**).** Overall effects of active treatment were better than the control regardless of presence of co-morbidity **(**p**=**0.003**).**

These effects were well observed with better responder to treatment regardless of presence of co-comorbidity at week 8 **(**p**=**0.09**)** and week 12 **(**p**=**0.025**)** as given in the **Table 6** overleaf.

Table 6 Venous Clinical Severity Score (VCSS). Responder -50 % Score Reduction (Based on Presence of Co-morbiity)

VCSS Total Score 50% Reduction	Control (N=21) Numbers with 50% Score reduction N/Total (%)	Active (N=21) Numbers with 50% Score reduction N/Total (%)	P-Value
After 4 weeks			
No Comorbidity	**1/16 (6.25%)**	9/17 **(52.94%)**	***P=0.007**
With Comorbidity	**1/5 (20.0 %)**	1/4 **(25.0%)**	***P=0.206**
All Patients	**2/21 (9.52%)**	12/21 **(57.14%)**	***P=0.003**
After 8 weeks			
No Comorbidity	2/16 **(12.50%)**	9/17 **(52.94%)**	***P=0.026**
With Comorbidity	1/5 **(20.0%)**	3/4 **(75.0%)**	***P=0.206**
All Patients	3/21 **(14.28%)**	12/21 **(57.14%)**	***P=0.009**
After 12 weeks			
No Comorbidity	3/16 **(18.75%)**	9/17 **(52.9%)**	***P=0.071**
With Comorbidity	1/5**(20.0%)**	3/4 **(75.0%)**	***P=0.206**
All Patients	4/21 **(19.04%)**	12/21 **(57.14%)**	***P=0.025**
All responder after 12 weeks	4/21 **(19.04%)**	12/21 **(57.14%)**	***P=0.025**

***P-value-Fisher's Exact**

Note. (Hyprtension 7/42, Musculoskeletal Disorder 2/42, No-comorbidity 33/42)

The effects reflected that active treatment provided better responder rate were also well observed with better responder to treatment regardless of both presence of co-comorbidity and CEAP severity combined at week 4 **(**p**=**0.004**)**, week 8 **(**p**=**0.009**)** and week 12 **(**p**=**0.032**)** as given in the **Table 7** overleaf.

Table 7 Venous Clinical Severity Score (VCSS). Responder -50 % Score Reduction (Presence of co-morbidity and Different Severity- CEAP Class1-2)

VCSS Total Score 50% Reduction	Control (N=21) Numbers with 50% Score reduction, N/Total (%)	Active (N=21) Numbers with 50% Score reduction, N/Total (%)	P-Value
After 4 weeks	With Co-morbidity		
CEAP Class 1	0/3 (0%)	1/2 (50.0%)	*P=0.400,
CEAP Class 2	1/2 (50.0%)	2/2 (100.0%)	*P=0.100
All CEAP 1+2	1/5 (20.0%)	3/4 (75.0%)	*P=0.206
	No Co-morbidity		
CEAP Class 1	0/5 (0%)	5/8 (62.5%)	*P=0.075
CEAP Class 2	1/11 (9.09%)	4/9 (44.44%)	*P=0.127
All CEAP 1+2	1/16 (6.25%)	9/17 (52.90%)	*P=0.007
All CEAP 1+2			P=0.004
After 8 weeks	With Co-Morbidity		
CEAP Class 1	0/3 (0%)	0.0%)	*P=0.075
CEAP Class 2	2/11 (18.18%)	1.44%)	*P=0.336
All CEAP 1+2	2/14 (14.28%)	5.45%)	*P=0.026
	No Co-morbidity		
CEAP Class 1	0/5 (0%)	2.5%)	*P=0.075
CEAP Class 2	2/11 (18.18%)	4.44%)	*P=0.336
All CEAP 1+2	2/16 (14.28%)	2.90%)	*P=0.026
All CEAP 1+2			*P=0.009

*P-value – Fisher's Exact

VCSS Total Score 50% Reduction	Control (N=21) Numbers with 50% Score reduction, N/Total (%)	Active (N=21) Numbers with 50% Score reduction, N/Total (%)	P-Value
Continue Table 7			
After 12 weeks	With Co-Morbidity		
CEAP Class 1	0/3 (0%)	1/2 (50.0%)	*P=0.400
CEAP Class 2	1/2 (50.0%)	2/2 (100.0%)	*P=1.000
All CEAP 1+2	1/5 (20.0%)	3/4 (75.0%)	*P=0.206
	No Co-Morbidity		
CEAP Class 1	0/5 (0%)	5/8 (62.5%)	*P=0.075
CEAP Class 2	3/11 (27.27%)	4/9 (44.44%)	*P=0.642
All CEAP 1+2	3/16 (18.75%)	9/17 (52.94%)	*P=0.071
All responder after 12 weeks	4/21 (19.04%)	12/21 (57.14%)	*P=0.032

*P-value – Fisher's Exact

Disability asssed as secondary objectives

4.2.5 Venous Clinical Severity Score (VCSS)-Total Score

It was found that the baseline Venous Clinical Severity Score (total) between the Control and Active treatment was not different (p=0.257) at Week 0 or baseline. Subsequent to follow-up assessment for treatment effects between Control and Active, patients treated by Active treatment tended to have better improvement assessed by venous clinical severity score after Week 4 (p<0.001), Week 8 (p<0.001) and Week 12 (p<0.001) tested by T-Test assuming normal distribution as compared between Control and Active, found that active treatment was better in improving overall symptoms assessed by reduction of venous severity clinical score at time In these respects, the Active treatments definitely provided better outcomes in terms of reduction of clinical severity assessed by Venous Clinical Severity Score as given in **Table 8**. overleaf.

Table 8 Venous Clinical Severity Score (VCSS). * Total Score

VCSS Total Sore	Control (N=21) Mean(SD), 95% CI,Min-Max	Active (N=21) Mean (SD), 95%CI Min-Max	*P-value
Day 0	27.2321 (6.4908), 24.2775 - 30.1850, 12.50-37.50	25.0000 (6.0917), 22.2271 - 27.7729, 15.63-34.38	*0.257
Week 4	22.4702 (7.9438), 18.8542 - 26.0862, 9.38-37.50	11.9048 (6.4908), 7.9673 - 15.8422, 0.00-31.25	*<0.001
Week 8	22.0238 (7.6849), 18.5257 - 25.5220, 9.38-37.50	17.1131 (8.3791), 8.3880 – 16.0168, 0.0-31.25	*<0.001
Week 12	21.7262 (7.4647), 18.3457 - 25.5220, 9.38-37.50	12.2024 (8.1432), 8.4956 - 15.9092, 0.0-31.25	*<0.001

* P-value T-test (Active vs Controlled)

4.2.6 Physician Rating Symptoms Perception Score (PRSPS)-Total

It was also founded that another assessment employing Physician Rating Symptoms Perception Score (PRSPS) (Total) observed with the total symptoms score from baseline between the Control and Active treatment was slightly different (p=0.010) at baseline. However, subsequent to follow-up assessment of treatment effects between Controlled and Active, patients treated by Active treatment tended to have better improvement assessed by Physician Rating Symptoms Perception Score (PRSPS) reduction after Week1 (p<0.001), Week 2 (p=0.001), Week 3(p<0.001), Week 4 (p=0.001), Week 8(p=0.001) and week 12 (p=0.002) were significant different as compared with Control treatment, In these respects, the Active treatments definitely provided better outcomes in terms of symptoms improvement assessed by Physician Rating Symptoms Perception Score (PRSPS) as given in **Table 9**. overleaf.

Table 9 Physician Rated Symptom Perception Score (PRSPS)-Total Score

PRSPS-Total Score	Control (N=21) Mean(SD), 95% CI,Min-Max	Active (N=21) Mean (SD),95%CI, Min-Max	*P-value
Day 0	42.9630 (7.0038)	37.1429 (6.8545),	0.010
	39.7749 -46.1511, 33.33 -57.78	34.0027 - 40.2630, 24.44-51.11	
Week 1	42.4339 (10.2222),	30.8995 (9.4256),	<0.001
	37.7555 - 47.1122,26.67 60.00	26.6090 - 35.1900, 20.00-60.00	
Week 2	39.7884 (9.8106),	29.5238 (8.1994),	0.001
	35.3226 - 44.2541, 24.44-55.56	25.7915 - 33.2562, 20.00-53.33	
Week 3	38.5185 (9.9711),	27.3016 (8.4952),	<0.001
	33.9797 - 43.0573,22.22-53.33	23.4346 - 31.1686, 20.00-51.11	
Week 4	38.4021 (9.9001),	26.7725 (8.0294),	0.001
	31.8958 - 40.9086, 22.22-53.33	23.0356 - 30.5094, 20.00-46.67	
Week 8	35.7672 (9.4256),	26.1376 (8.4006),	0.001
	31.4767-40.0577, 22.22-51.11	22.3137 – 29.9615, 20.00-40.89	
Week 12	35.8730 (10.7562),	25.9259 (8.0226),	0.002
	30.9768 - 40.7692, 22.22-53.33	22.2741 - 29.5778, 20.00-48.89	

*P-Value T-test (Active VS Controlled)

4.2.7 Patients Self-Rating Symptoms Score (PSSS) -Total

Moreover, for patients self-reported by employing visual analogs sacle(VAS) assessment, **Patient Self-Rating Symptoms Perception Score (PSSS)** observed with the total symptoms score from baseline between the Control and Active treatment was not statistical significant different (p=0.118) at baseline. However, subsequent to follow-up assessment of treatment effects between Control and Active, patients treated **by Active treatment tended to have bettere improvement assessed by Patient Self-Rating Symptoms Score (PSSS) reduction after Week1 (p=0.006), Week 2 (p=0.005), Week 3(p=0.004), Week 4 (p=0.004), Week 8(p=0.003) and week 12 (p=0.003) were significant different as compared with Control treatment.** In these respects, the **Active treatments definitely provided better outcomes in terms of symptoms improvement self-assessed by Patient Self-Rating Symptoms Score(PSSS)** as given in **Table 10**. overleaf.

Table 10 Patient Self-Rated Symptom Score (PSSS). Total Score

PSSS Total Score	Control (N=21) Mean(SD), 95% CI, Min-Max	Active (N=21) Mean (SD), 95% CI,Min-Max	*P-value
Day 0	35.2585 (8.0086),	31.7211 (6.2347),	
	27.2499 - 43.2671, 30.86-50.57	25.4864 - 37.9558, 26.71-38.57	P=0.118
Week 1	24.8980 (7.4151),	19.2449 (5.0210),	
	17.4831 - 32.4041, 26.71-38.57	14.2239 - 24.2659, 12.14-31.14	P=0.006
Week 2	23.0680 (7.7269),	17.1020 (4.8117),	
	15.3411 - 30.7949, 26.86-37.29	12.2903 - 21.9137, 17.57-28.29	P=0.005
Week 3	23.0816 (7.7273),	16.9864 (5.0209),	
	15.3543 - 30.8089, 26.86-37.14	23.4346 - 31.1686, 19.57-29.43	P=0.004
Week 4	22.9524 (7.7321),	16.8639 (4.9864),	
	15.2203 - 30.6845, 26.86-36.86	11.9655 - 21.8503, 19.57-29.43	P=0.004
Week 8	22.9592 (7.6331),	16.7347 (4.7568),	
	15.3261 - 30.5923, 27.57-36.86	11.9779 – 21.4915, 18.57-28.14	P=0.003
Week 12	22.8571 (7.5951),	16.6803 (4.6080),	
	15.2620 - 30.4522, 25.86-35.86	12.0723 - 21.2883, 19.00-29.00	P=0.003

***P-Value T-test (Active Vs Controlled)**

4.2.8 Medical Outcomes Stydy-12-Items Short-Form Health Survey (MOS SF 12)

The analysis compared all Physical Component Summary score (PCS), Mental Component Summary score (MCS) and global score of MOS SF 12 score between Active and Control treatment both at baseline and after 12 week of treatment, then the analysis compared impact of overall treatment effecte (Before and After treatment –Baseline and Week 12)

Moreover, for an overall general health-related quality of life aspects by employing structure questionnaire a Medical Outcomes Study, 12-item Short-Form Health-Survey (MOS SF-12) assessment, from baseline (Day 0) between the Control and Active treatment, for there were no significantly different between each category of score between Active and Control for PCS score (p=0.860), MSC score (p=0.257) and Global Score (p=0.427) for baseline (day 0) given **in Table 11** and also no significantly different between each category of score between Active and Control for PCS score (p=0.809), MSC score (p=1.000) and Global Score (p=0.874) for baseline (week 12) given **in Table 12** overleaf.

Table. 11 Medical Outcomes Study 12-Items Short-Form General Health Survey (MOS SF 12) –Physical Component Summary Score (PCS), Mental Component Summary Score (MCS) and Global Score (At baseline- Day 0)

MOS -Item SF 12	Control (N=21) Mean (SD),Min-Max,Range	Active (N=21) Mean (SD)l,Min-Max,Range	P-value
PCS	84.6032 (3.06887), 76.67 – 90.00,13.33	84.7619 (2.7021), 80.00 -90.00,10.00	0.860
MCS	86.1905 (2.4234), 76.67 -90.00,13.33	86.8254 (0.7273), 86.67 - 90.00,3.33	0.257
Global Score	85.3965 (1.8184), 81.67 – 88.33,6.67	85.7937 (1.3559), 83.33 -85.33, 5.00	0.427

*P-Value T- Test

Table. 12 Medical Outcomes Study 12-Items Short-Form General Health Survey (MOS SF 12) –Physical Component Summary Score (PCS), Mental Component Summary Score (MCS) and Global Score (At the end of trial- Week 12)

MOS -Item SF 12	Control (N=21) Mean (SD),Min-Max,Range	Active (N=21) Mean (SD)l,Min-Max,Range	P-value
PCS	92.3810 (2.3904), 86.67 – 93.33, 6.67	92.5397 (1.7965), 86.67 -95.33,6.67	0.809
MCS	86.3492 (2.5614), 80.00 -93.33,13.33	86.8492 (1.4547), 80.00-86.67,6.67	1.000
Global Score	89.361 (2.0052), 83.33 – 93.33,10.00	89.4444 (1.0971), 86.67 -90.00, 3.33	0.874

*P-Value T- Test

However, after 12 week of follow-up, a Medical Outcomes Study, 12-item Short-Form Health Survey (MOS SF-12) assessment for both Control and Active treatment, for PCS, MCS and Global score of MOS SF-12 were statistically improvement (p<0.001) for PCS, Global Score(p<0.001) but not for MCS (P=0.496 and P=0.245) -in Table 13.

Table. 13 Medical Outcomes Study 12-Items Short-Form General Health Survey (MOS SF 12) –Physical Component Summary Score (PCS), Mental Component Summary Score (MCS) and Global Score (Control Treatment– Baseline – the end of trial -Week 12)

MOS –Item SF 12	Control (N=21)-Day 0 Mean (SD), Min-Max,Range	Control (N=21)- Week 12 Mean (SD), Min-Max,Range	P-value
PCS	84.6032 (3.06887), 76.67 – 90.00,13.33	92.3810 (2.3904), 86.67 – 93.33,6.67	<.0.001
MCS	86.1905 (2.4234), 76.67 -90.00,13.33	86.3492 (2.5614), 80 – 93.33,13.33	0.496
Global Score	85.3965 (1.8184), 81.67 – 88.33,6.67	89.3651(2.0052), 83.33 -93.33, 10.00	<0.001

(Active Treatment – Baseline - the end of trial- Week 12)

MOS –Item SF 12	Active (N=21)-Day 0 Mean (SD),Min-Max,Range	Active (N=21)-Week 12 Mean (SD),Min-Max,Range	P-value
PCS	84.7619 (2.7021), 80.00 -90.00,10.00	92.5397 (1.7965), 86.67 – 93.33,6.67	<0.001
MCS	86.8254 (0.7273), 86.67 - 90.00,3.33	86.3492 (1.4547), 80 – 86.67,6.67	0.245
Global Score	85.7937 (1.3559), 83.33 -85.33, 5.00	89.4444 (1.0971), 86.67 -90.00, 3.33	<0.001

*P-value Paired T-Test

4.2.9 Medical Outcomes Stydy-14-Items Short-Form Health Survey for Chronic Venous Insufficiency (**MOS CIVIQ14**)

The analysis compared for the pain score, physical function score, psychological and social function score and global score between Active and Control treatment both at baseline and after 12 weeks for both control and active treatment, then the analysis compared impact of overall treatment effecte **(**Before and After treatment –Baseline and Week 12**)**

From baseline **(**Day 0**)** between the Control and Active treatment, for the pain score, physical function score, psychological and social function score and global score for both control and active treatment were not significantly different at baseline both for Active and Controlled **(**p**=**0.432, p**=**0.153, p**=**0.576 and p**=**0.511**)** given in **Table 14** overleaf**.**

From end of treatment **(**Week 12**)** between the Control and Active treatment, for the pain score, physical function score, psychological and social function score and global score for both control and active treatment were also not significantly different after the week-12 for both the Active and Controlled **(**p**=**0.213, p**=**0.702, p**=**0.115 and p**=**0.645**)** given in **Table 15** overleaf**.**

Table. 14 Medical Outcomes Study 12-Items Short-Form General Health Survey (MOS CIVIQ 14) –Pain, Physical Function, Psychological and Social Function and Global score (At baseline- Day 0)

MOS CIVIQ 14	Control (N=21) Mean (SD), Min-Max,Range	Active (N=21) Mean (SD), Min-Max,Range	P-value
Pain	61.4286(8.0843), 45.00 -75.00,30.00	63.5714 (9.3732), 45.00 – 75.00,30.00	0.432
Physical Function	58.7755(4.9597), 49.29 – 66.43,17.14	56.4286(5.4772), 49.29 -66.43, 17.14	0.153
Psycholo- & Social Function	60.3571 (5.2013), 47.50 – 70.0022.50	61.3095 (5.7347), 52.50 -75.22, 5.00	0.576
Global Score	68.6905 (4.3025), 60.00 – 75.00,15.00	70.4762(2.6947), 65.00- 75.00,10.00	0.511

*P-value T-Test

Table. 15 Medical Outcomes Study 12-Items Short-Form General Health Survey (MOS CIVIQ 14) –Pain, Physical Function, Psychological -Social Function and Global score (After Week 12)

MOS CIVIQ 14	Control (N=21) Mean (SD), Min-Max,Range	Active (N=21) Mean (SD), Min-Max,Range	P-value
Pain	67.8571 (7.6764), 60.00 – 75.00,15.00	70.7143 (6.9436), 60.00 -75.00,15.00	0.213
Physical Function	67.3469 (3.8075), 60.00 – 75.00,15.00	66.9388 (3.0232), 64.29 -75.00, 10.71	0.702
Psycholo- & Social Function	68.6905 (4.3025), 60.00 – 75.00,15.00	70.4762 (2.6947), 65.00 -75.00, 10.00	0.115
Global Score	79.52 (5.5700), 68.57 – 91.43,	78.70 (5.8000), 76.06- 81.34	0.645

*P-value T-Test

Moreover, for an overall health-related quality of life aspects impacts from baseline (day 0) and after completion (Week 12) for both the Control and Active treatment for the pain score, physical function score, psychological and social function score and global score for both control and active treatment were not significantly different at baseline were significantly improved for the Active and Controlled ($p<0.001$, $p<0.001$, $p<0.001$ and $p<0.001$) both group respectively, the final HRQOL Score after 12-weeks were all improved and significsantly better than at the initiation of the trial as given in **Table 16 and Table 17** overleaf.

Table. 16 Medical Outcomes Study 14-Items Short-Form Chronic Venous Disease Survey (MOS CIVIQ 14) –Pain, Physical Funciton, Psychological-Social Function and Global Score (Control Treatment– end of trial Week 12 - Baseline –Day 0)

MOS CIVIQ 14	Control (N=21)-Day 0 Mean (SD),Min-Max,Range	Control (N=21) Week 12 Mean (SD),Min-Max,Range	P-value
Pain	61.4286(8.0843), 45.00 -75.00,30.00	67.8571 (7.6764), 60.00 – 75.00,15.00	<0.001
Physical Function	58.7755(4.9597), 49.29 – 66.43,17.14	67.3469 (3.8075), 60.00 – 75.00,15.00	<0.001
Psycholo- & Social Function	60.3571 (5.2013), 47.50 – 70.0022.50	68.6905 (4.3025), 60.00 – 75.00,15.00	<0.001
Global Score	68.6905 (4.3025), 60.00 – 75.00,15.00	79.52 (5.5700), 68.57 – 91.43,	<0.001

***P-value Paired T-Test**

Table. 17 Medical Outcomes Study 14-Items Short-Form Chronic Venous Disease Survey (MOS CIVIQ 14) –Pain, Physical Funciton, Psychological-Social Function and Global Score (Active Treatment the end of trial -Week 12– Baseline – Day 0)

MOS CIVIQ 14	Active (N=21)-Day 0 Mean (SD),Min-Max,Range	Active (N=21)- Week 12 Mean (SD)I,Min-Max,Range	P-value
Pain	63.5714 (9.3732), 45.00 – 75.00,30.00	70.7143 (6.9436), 60.00 -75.00,15.00	<0.001
Physical Function	56.4286(5.4772), 49.29 -66.43, 17.14	66.9388 (3.0232), 64.29 -75.00, 10.71	<0.001
Psycholo- & Social Function	61.3095 (5.7347), 52.50 -75.22, 5.00	70.4762 (2.6947), 65.00 -75.00, 10.00	<0.001
Global Score	70.4762(2.6947), 65.00- 75.00,10.00	78.70 (5.8000), 76.06- 81.34	<0.001

*P-value Paired T-Test

4.3 Adverse Drug Events reported

The investigation demanded subjective response in terms of should incidence of adverse drug events **(**if any**)** occurred during a weekly follow**-**up period reported as spontaneous report by patients during the trial**.** This was due basically to an early pilot study of the same in healthy volunteer of this formulations reflected nonsystematic adverse drugs event which were external skin reaction **[**158**].** Mostly uncomfortable skin reactions**.** As such, this reports were summary reports as given in **Table 18** overleaf**.**

Table. 18 Report of Adverse Event over 12-weeks period of follow-up.

Summary Period	Control (N=21) Number, (%)	Active (N=21) Number, (%),	P-Value
Week 4	No 7/21 (33.3 %), Some-Tolerate 14/21 (66.7%)	No 7/21 (33.3 %), Some-Tolerate 14/21 (66.7%)	0.628
Week 8	No 12/21 (57.4 %), Some-Tolerate 9/21 (42.6 %)	No 14/21 (66.7 %), Some-Tolerate 7/21 (33.3%)	0.376
Week 12	No 20/21 (95.1 %), Some-Tolerate 1/21 (4.9%)	No 18/21 (85.7 %), Some-Tolerate 4/21 (14.3%)	0.756

P-value Fisher's Exact Test

CHAPTER V

DISCUSSIONS AND CONCLUSIONS

5.1 DISCUSSIONS

This study, employed the ACTIVE treatment (Herbal Medicine Combination – containing 2% Asiaticoside plus 1% Acemannan) –formulated in a 2% Acetyl Salicylic Acid in Beeswax encapsulation gel base, whereas the CONTROL treatment contained only a 2% Acetylsalicylic Acid in Beeswax encapsulation gel base preparation. During the pre-formulation phase, the encapsulation of Acetylsalicylic Acid in Beeswax was employed as the test model for study the release of Acetylysalicylic Acid from the encapsulation per se, in spite of investigation for the release of Asiaticoside and Acemannan from the formulation. This was due basically to two main assumptions as agreeable, firstly Asiaticoside is water soluble extracts similar to Acetylsalicylic Acid with the unavailable pure standard of Asiaticoside to allow precise absorption curve to study the release, secondly the Acemmannan, though as water soluble, the intended therapeutics is externally protective for the skin in the formulation rarther than the penetration of the substance to circulation even though higher contents of short to moderate molecular chain (<15 Daltons) Acemannan was used.

The patient recruitment, a screening process during pre-enrollment follow-up had been encountered some difficulty as early as the trial. As such with further consultation, investigator decided not to compromise and follow per protocol investigation. As such, the sample size as estimated was deficit and less than anticipated. Therefore, numbers of patients undergone randomization were less than

as pre-assumption. Such that, only the final analysis could be conducted as per protocol analysis. The sample size estimation was due basically to the assumption of possible responder to the control/placebo treatment could be as high as 40%, though this study had reflected only confirmed that control/placebo treatment as low as only 9.52%. Since sample size estimation is demanding as an early stage of conducting a clinical study, in reality when different investigations conducted with the same methodology and securing similar outcomes only with different sample size, may point this research in different directions to making clinical decisions. As such, samples should not be too small as well as excessive. **Though the objective of this study was to test whenther the ACTIVE treatment provides better responder rate than the CONTROL treatment in a set hypothesis**. As such, in our real scenario, the test hypothesis as implicated though significantly demonstrated that ACTIVE treatment provided better responder rate than the CONTROL treatment and responder rate was depependent on the ACTIVE intervention, **at a given point there was less power to conclude as inadequate sample size as indicated**. However, in the light of this, **the responder rate** (**with smaller sample size**) **as increases the chance of assuming as true at a false assumption**. In other words, responder rate of the ACTIVE treatment may not be superior to CONTROL treatment suggesting re-testing for another appropriate assumption on the control responder rate. However, vice versa since in reality the responder rate of the CONTROL treatment was far lower than that estimated as observed in this trial (9.5% VS 40%), then the sample size calculation was probably over-estimation at the given power of 80%. In such circumstance the over responder rate of 40% of the control/placebo as predetermined may need to rejustify if such treatment combined other factors such

as co-medication or other confounding factors that may in fact affect the efficacy of control/placebo treatment. Unlike, the above our assumption should be readjusted.

This study was an original investigation with attempt to alleviate and delay progression of chronic venous disease in clinical practice using herbal medicine as combination with probable evidence from early other herbal extracts study**.** Those possible herbal extracts such as Asiaticoside, Horse-Chestnut Seed Extract (HCSE), Extract of a French Maritime Pine Bark (Pycnogel®), Micronized Purified Flavonoid Fraction (MPFF), Ginkor Fort, Buckwheat Herb Tea, Red-Vine-Leaf Extract (RVLE), Chinese herbal medicine and even Acetylsalicylic Acid (ASA) had been reported despite lack of definitive conclusion due to study designs and recruitment of sample patients. Despite such limitation, the so-called **Herbal Medicine Combination (HMC)** in this study had combined possible beneficial effects of such ingredients into one formulation, and thus to minimize risk and optimize benefits for chronic venous disease patienst with leg symptoms. Thereby, the innovative pharmaceutical methods for encapsulation of Acetylsalicylic Acid with Beeswax was adopted, at least to double down possible risk for oral administration. Early investigations of herbal medicine formulation, many of which were conducted as in an oral preparation such as **Horse Chestnut seed extracts** [**Ernst E., 1999**], where authors suggested that this extract could alleviate subjective symptoms and **reduce the objective signs of chronic venous insufficiency** [110]. The **Red vine leaf extract (RVLE) [Kiesewetter, H et al, 2000**] likewise, authors suggested that the oral administration may **help alleviate stage I/stage II chronic venous insufficiency but only subjective symptoms improvements** were significantly better as compared with

placebo [111]. The **oral horse chestnut seed extract and aloe vera gel** [**Ernst, E et al, 2002, Morling, Joanne et al, 2013] for which authors proposed should be effective in relieving symptoms of chronic venous disease,** the authors suggested that this **extract may be effective but need to be cautious due to certain risks of advere drug events** [112, 120]. The **Pygnogenol (PYC), extracts form grap seed** containing procyanidins and phenolic acids [**Rohdewald, P et al, 2002, Schoones A., et al, 2012],** the authors also suggested this **may be effective for treatment of chronic venous insufficiency by its anti-oxidative stress activity** [113,119] which cannot demonstrate definitively in clinicql setting. **The horse-chestnut seed extracts, aescin, rutin, troxerutin, diosmin and hesperidine [Bylka, Wieslawa, et al 2005] as oral administration**, the authors suggested **that the extracts could alleviate symptoms of chronic venous disease though still need further investigation** [114]. The **Topical Traditional Chinese Medicaments (TTCM**), **[Lim, Kar Seng et al, 2007],** also suugested the topical TTCM as pathes could be **effective in patients with chronic venous leg ulcers but may cause sensitivity to skin** [115]. **The bark of horse chestnut extract** [**Felixsson, Emma et al 2010], for which** the authors suggested **oral administration of the preparation of these extracts could be effective for treatment of chronic venous insufficiency** [116]. **An acidic glycoconjugate from Lythrum salicaria L with the effect on blood coagulation [Pawlaczyk, Izabela et al, 2010]** suggested its may be effective for treatment of varicose veins, and venous insufficiency as due basically to traditional use of Lythrum salicaria as a pro-coagulant activity [117]. **For Asiaticoside**, [**Pointel JP, et al 1987],** the authors conducted a study among 94 patients suffering from venous insufficiency of the lower limbs as a multicenter, double-blind versus placebo study for two-

month oral administration, authors reported a significant difference ($p < 0.05$) in favor of Asiaticoside for improvement of **symptoms of leg heavy, leg edema and satisfactory for overall evaluation by the patient**. [144]. Another authors [**Cesarone MR et al, 1994, 2001]** conducted a prospective, randomized trial looking at efficacy of an oral preparation of Asiaticoside in the microcirculation and reducing leg edema (leg volume) among fourty patients suffering from venous microangiopathy patients with venous hypertension among 24 patients for each group was prescribed randomly with either a 60-mg tablet of TECA or placebo, twice daily for 6 weeks as a pre-and-post treatment comparison, after treatment there was a decrease in resting flux (29%) and an improvement (increase) in venoarteriolar response (52%); PO2 was increased (7.2%) and PCO2 decreased (9.6%). However, there was **no proper controlled or direct comparison between placebo and active treatment groups** [145,146]. Another investigation of the same, [**Belcaro G et al, 2001]** assessed effects of oral Asiaticoside 60-mg tablets taken twice daily for 4 weeks amomg 50 patients looking at the rate of ankle swelling (RAS) in patients with ankle edema due to venous hypertension before and after treatment. The authors reported and suggested significant improvement with 34% reduction in rate of ankle swelling as compared with before treatment in chronic venous insufficiency could start as early as development of leg edema to control deterioration leading to leg ulcerations. Authors suggested the complex actions on the microcirculation was essential for reducing edema [148]. **Enck, Paul et al, 2011 [**149**]** suggested that there was a certain degree of placebo and drugs response in clinical trial which could range from 32**%** to 52**%** depending on the type of designed and both **Kirsch, I and LJ Miller, 1988, 2005 [**150,151**]** suggested that event though the double-blinded

approach to abolish bias in placebo-response, the way administration of a placebo by elaboration to the participants could emerge with deceptive placebo response.

As above, this study should be the original investigation different from the above. This study conducted in chronic venous disease mild to moderate patients to overcome research gaps in the various studies due to (1) **a design as a double-blind randomized controlled trial and adding the possible standard controlled as such (**in this formulation as Acetylsalicylic Acid**)**, (2) **avoid the possible systematic risks of oral absorption** and (3) **conducted in Chronic Venous Disease mild-to-moderate severity diagnosed by specialists** which were more specific. The overall primary objective or endspoints was the responder to treatment **(**responder rate**)** as defined by reduction of 50**%** of Venous Clinical Severity Score after one month or 4 weeks of treatment comprising the ACTIVE and CONTROL treatment.

From the Hypothesis Testing (as the primary endpoints)

The Null Hypothesis H_0 = The responder to treatment is independent to Active intervention and **to reject Null Hypothesis if p<0.05.,** thereby accepted the Alternative Hypothesis.

The alternative Hypothesis H_1 = The responder to treatment is dependent on the Active intervention.

From this investigation, both the ACTIVE Treatment an CONTROL Treatment successfully reduced clinical severity by improvement of the overall mean**(**SD**)** score of Venous Clinical Severity Score **(**VCSS**)** total score from baseline **(**Day 0**)** for ACTIVE 25.00 **(**6.0917**)** to 11.9048 **(**6.4908**)** as compared with for the CONTROL 27.2321**(**6.4908**)** to 22.4702 **(**7.9438**)** was not different at baseline **(**p=0.257**).**

However, after 4 weeks of treatment the overall VSCC from Active treatment of 12.2024 (8.1432) as compared with Control treatment of 21.7262 (7.4647) respectively was highly significant different where ACTIVE treatment was better improvement of the over all clinical severity assessed by Venous Clinical Severity Score (p<0.001). The primary objective for overall responder rate of the ACTIVE treatment after 4 weeks for ACTIVE of 12/21 (57.14%) as compared with for the CONTROL of 2/21 (9.52%) after 4 weeks of treatment was significantly better (p=0.003). The same for continuation of treatment after 8 and 12 weeks with ACTIVE treatment rendered better improvement of th over all symptoms by reducing the severity assessed by Venous Clinical Severity Score (P<0.001) improvement overtime as assessed by score reduction, which were significant from basline to week 4, week 4 to week 8 of 11.9048(6.4908) VS 22.4702(7.9438) , p<0.001 with responder at 12/21 (57.14%) VS 3/21(14.28%), p=0.009 and week 8 to week 12 of 17.1131(8.3791) VS 22.0238(7.6849), p<0.001 with responder rate at 12/21 (57.14%) VS 4/21(19.04%) p=0.025 (asessed by T-test assuming normal distribution, and Fisher's Exact Test) (Table 4 and Table 8), in short both the ACTIVE and CONTROL reduced clinical severity though the extent of ACTIVE treatment was far better than the CONTROL.

This investigation founded that responder rate to ACTIVE treatment was better than CONTROL treatment regardless of all CEAP Class 57.149% VS 9.52%), p=0.003, the milder class CEAP Class 1 was better 60.0% VS 0%, p=0.013 (Table 5). The responder rate was better and regardless of presence of co-morbidity 57.14% VS 9.52%, p=0.003 whereas the Active treatment in with no comorbidity provided better responder rate 52.9% VS 6.25% (p=0.007) (Table 6). The CONTROL responder

rate was somewhat lower than anticipation as our assumption, for control responder rate at 40**%** for which ACTIVE treatment should be 35**%** superior to CONTROL to be significant different**.** As such since our sample size calculation was compromised by actual lower control responder rate whereby Active treatment responder rate is over 35**%** responder rate higher than control treatment**.** These responder rate was significantly dependent on the ACTIVE intervention**. (**p**-**value, Chi**-**Square Test and Fisher's Exact Test**.) (**Table 4 to 7**).**

For the secondary endpoints analysis, the improvement as observed by Venous Clinical Severity Score **(**VCSS**)**, the Physician Rate Symptoms Perception Score **(**PRSPS**)** in this respects were obtained by clinical rated **(**VCSS**)** and a direct structured enquires, the same for Patient**-**Self**-**Rated Sympotms Score **(**PSSS**)** as given in Table 9**/**10**.** These results reflected the overall improvements were observed across the board, and were dependent on the ACTIVE intervention **(**all reflect p<0.05**) (**Table 8 to Table 10**).**

For the Health**-**Related Quality of Life both assessed by the disease specific, a chornic venous disease questionnaires scale **(**MOS CIVIQ14, with subscores of Pain, Physical function, Psychological and social functions, Global Score**)** and general health questionnaires scale **(**MOS SF12, with the subscore for physical component summay, PCS and mental component summary, MCS and global score**)** were all reflected similar improvement from baseline to after 12 week of treatment, and observing similarly by both treatment and were dependent on both ACTIVE and CONTROL treatment for all aspects of the score reported**.** It is to be notified, only

that the mental component summary **(**MCS**)** MOS SF 12, did not significantly improved subsequent to treatment for both the control and the active group**.** This may due to subjective response with small sample size may be difficult to interpret the individual symptoms score unlike the clinical score which was clinically assessed**.** However, there was no statistical signifinat different in terms of the overall health-related quality of life assessed between the two ACTIVE and CONTROL treatment for all the two MOS CIVIQ 14 and MOS SF 12 and their subscore**.** It was probably due to their less sensitivity of the scales for both to detect minor different for chronic venous disease symptoms domain**.**

Furthermore, the analysis of the safety looking at frequency of reported adverse events as compared between the ACTIVE and CONTROL treatment reflected no statistical significant different between the outcomes observed for both groups in terms of the reported advere events**.** As above, the summary of spotted reports from patients' viewpoints were no statistical significant different of adverse events reported irrespective of individual patients as given in Table 18**.**

5.2 CONCLUSIONS

From this investigation, as from the hypothesis testing and anticipated sample size of 60 to see the different of 35**%** responder rate, this investigation suggested, base on minor limitation and the evidence above, that the **Herbal Medicine Combination containing 2% Asiaticoside in 1% Acemannan and 2% Acetylsalicylic Acids** (**As an ACTIVE TREATMENT**) **was more effective than the 2% Acetyl Salicylic Acids** (**As CONTROL TREATMENT**) **based on various assessment criteria reflected in the**

primiary endpoints which provides the responder rate of 57.14% as compared with 9.52% after 4 weeks of treatment (p=0.003) **regardless of clinical severity** (p=0.003) **and co-morbidty** (p=0.003). We rejected the null hypothesis and therefore accept the alternative hypothesis that the better responder rate was due to ACTIVE Treatment intervention**.** However, our sample size to observe this different under the primary assumption that sample size should be 60 or n=30 for each arms to detect the different of 35**%** better of ACTIVE than control may be true as a false assumption though was in limitation of our capacity due to deficit patient recruitment, screening and randomization as lower than anticipated**.** Nevertheless, a combination of the herbal medicine could be alternative complimentary in single innovative formulation to enhance clinical efficacy of a single substance in the formulation**.**

Overall safety asssement as available data on effects frequency of adverse event reports, there were as our limit data, we may declare that there may be no different between the two treatments in this short-term trial**.**

5.3 LIMITATION OF THE STUDY

This research was performed in a hospital setting at Chiang Rai Prachanukroh Hospital, and Somdet Phra Yanna Sangworn (SDPY) Hospital Wiang Chai, and Chiang Rai, Hospital Thailand. These patients sample inherited with specific lifestyle, culture and characteristics pertaining to food consumption, self-medication behavior and other uncontrollable local health behavior. They may not represent the national or global population. Though the study may assume that response to active treatment

with 40% CONTROL responder rate given 35% better responder rate as compared with active treatment is quite overwhelming controll responder. This study found that the control responder rate was categorically as low as 10%.

5.4 BENEFITS OF THE RESEARCH APPLICATION

This study inspired by local demand for herbal medicine for early management of chronic venous disease, the most common health complaint globally including Thailand. Since modern drug could not warrant complications should arise in long-term, whereas there is no standard medical treatment available. Moreover, the expensive cost of modern drugs or surgical tools for intervention could jeopardize the freely access to medication. The results of this investigation should foster further development for better formulation and promotion of the use of herbal plants and promotion as economic plants

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APPENDIX

Appendix 1

Herbal Extract Sources/Analysis

Asiaticoside (Freezed Dried - China/Acemannan (Freezed Dried, USA)

西安品诚生物科技有限公司
Xi'an Pincredit Bio-tech Co.,Ltd

PROFORMA INVOICE			
ADD: Room 22005, Unit 2,Shuzi Building 1, Technology Five Road 8, Hi-tech District, Xi'an, Shaanxi,710065			
Tel: 86 13259969727	**Fax:** 86(29)89198270		
NUMBER: PC-CI-15-01-19	**Date:**15-01-19		
To: College of Public Health Science, Chulalongkorn University	**ADD:**Institute Building 3 (10th -11th Floor), Chulalongkorn Soi 62, Phyathai Road, Bangkok 10330. Thailand		
Tel: 6682-5776754	**Contact Person:** C/O Dr. Naowarat Kanchanakhan		
Product name and Specification	**Quantity (KG)**	**Unit Price (USD)**	**Total Amount (USD)**
Gotu kola extract 40% asiaticoside	5	252	1260
Paypal Charge fee			40USD
Freight via DHL for 5kg to Thailand			**97USD**
Total Price			**1397USD**

Payment term:

T/T bank account
BENEFICIARY CUSTOMER: Feng Tao
Name of bank: Bank Of China Shaanxi Branch Xian Tang Yan Zhong Lu Sub-branch
Account number: 6217853600004736590
Address: 35# TANG YAN LU,XI' AN,SHAANXI.P.R CHINA
Swift code: BKCHCNBJ620

Delivery time: Dispatch within 5 working days after reception payment

Beneficiary's Information:

陕西省西安市高新区科技五路 8 号数字生活第 1 幢 2 单元 20 层 22005 室 710065
电话：+86-29-89198270 传真：+86-29-89198270
Room 22005, Unit 2,Shuzi Building 1, Technology Five Road 8, Hi-tech District, Xi'an, Shaanxi,710065
T：+86-29-89198270 F：+86-29-89198270

Analysis of Asiaticoside 40%

三为生物

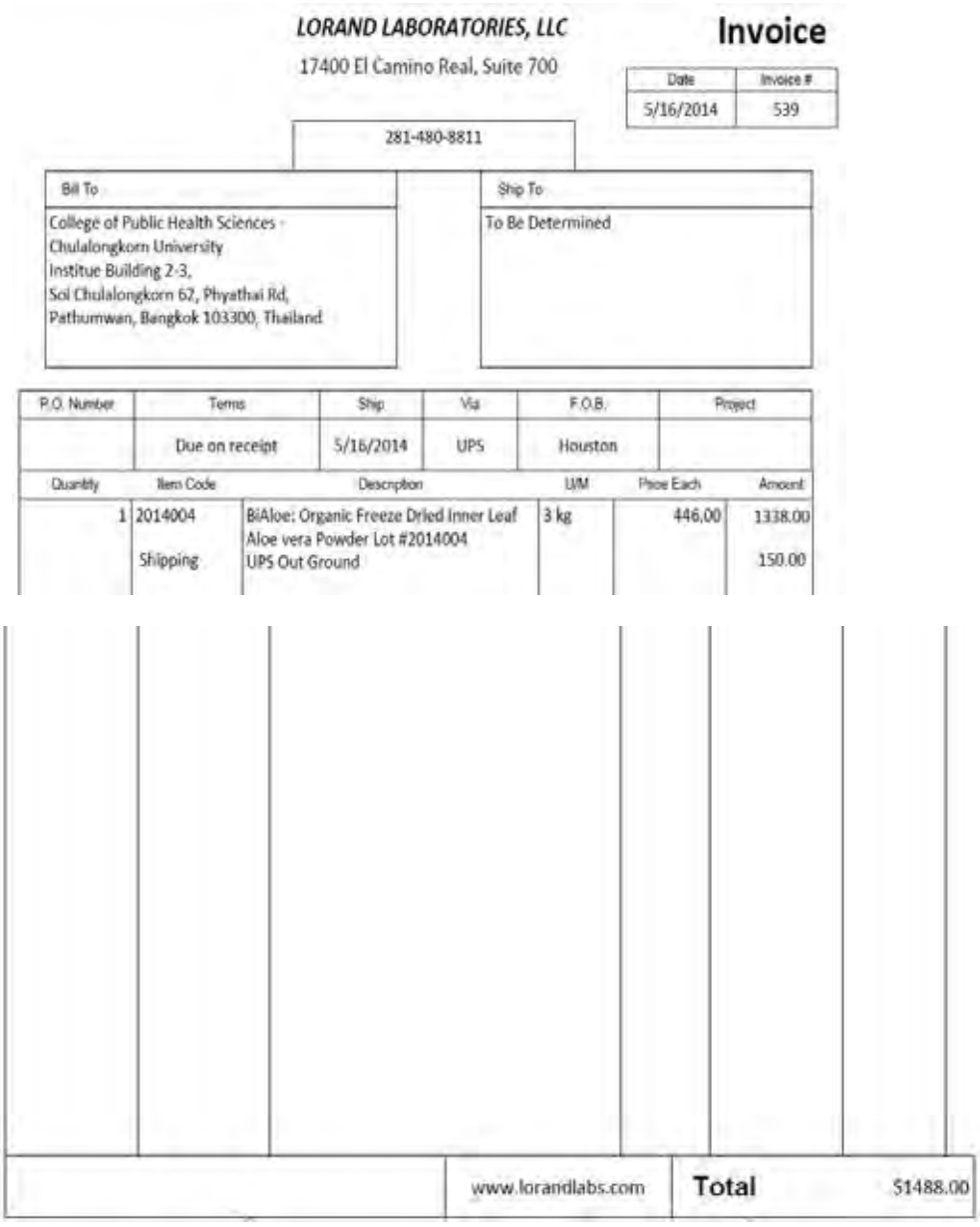
3W Botanical Extract Inc.

Http://www.3wbio.com

CERTIFICATE OF ANALYSIS

Product and Batch Information			
Product Name:	Gotu Kola Extract	**Country of Origin:**	P. R. China
Botanic Name:	*Centella Asiatica(L.) Urban*	**Type of extraction:**	Water&Ethanol
Plant Part:	Leaf	**Manufacture Date**	2014-5-22
Batch No:	GKE-140522	**Analysis Date**	2014-5-27
Batch Qty.:	28Kg	**Report Date**	2014-5-30

Assays	Specification	Result	Test Method
Active Ingredients			
Assays(HPLC)	NLT Asiaticoside 40%	40.56%	HPLC
	Total triterpenes ≥75% (asiaticoside&madecassoside)	Asiaticoside:40.68% Total triterpenes 70.54% (asiaticoside&madecassoside)	
Physical Control			
Identification	Positive	Complies	TLC
Appearance	Light yellow to milkiness powder	Complies	Visual
Odor	Characteristic	Complies	Organoleptic
Taste	Characteristic	Complies	Organoleptic
Sieve Analysis	100% pass 80 mesh	Complies	80 Mesh Screen
Loss on Drying	<5%	1.94%	5g / 105 ℃ / 2hrs
Ash	<5%	0.61%	2g / 525 ℃ / 5hrs
Chemical Control			
Arsenic (As)	NMT 1ppm	Conforms	Atomic Absorption
Cadmium(Cd)	NMT 1ppm	Conforms	Atomic Absorption
Lead (Pb)	NMT 1ppm	Conforms	Atomic Absorption
Heavy Metals	10ppm Max	Complies	Colorimetric method
Microbiological Control			
Total Plate Count	<10000cfu/g	Complies	Heat & Steam
Yeast & Mold	<1000cfu/g	Complies	Heat & Steam
Salmonella	Negative	Complies	FDA BAM 8th Ed.
E.Coli	Negative	Complies	FDA BAM 8th Ed.
Packing and Storage			
Packing	25kg/Drum, Pack in paper-drums and two plastic-bags inside.		
Storage	Store in cool dry area. No freezing. Keep away from strong direct light and heat.		
Shelf Life	2 years if sealed and store away from direct sun light.		
Expiration Date	2016-5-21		



LORAND LABORATORIES, LLC

17400 El Camino Real, Suite 700

281-480-8811

Invoice

Date	Invoice #
5/16/2014	539

Bill To	Ship To
College of Public Health Sciences Chulalongkorn University Institue Building 2-3, Soi Chulalongkorn 62, Phyathai Rd, Pathumwan, Bangkok 103300, Thailand	To Be Determined

P.O. Number	Terms	Ship	Via	F.O.B.	Project
	Due on receipt	5/16/2014	UPS	Houston	

Quantity	Item Code	Description	U/M	Price Each	Amount
1	2014004	BiAloe: Organic Freeze Dried Inner Leaf Aloe vera Powder Lot #2014004	3 kg	446.00	1338.00
	Shipping	UPS Out Ground			150.00

www.lorandlabs.com

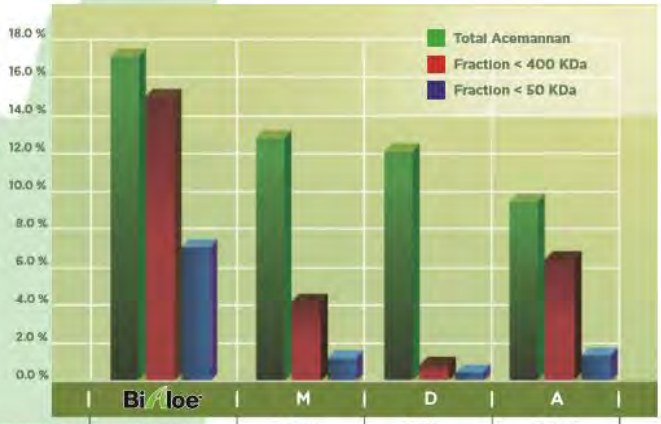
Total $1488.00

LorAnd LABORATORIES™ *The Perfection of Aloe Vera Processing™*

BiAloe® Highest Quality Aloe Vera Made

Aloe vera L (Aloe barbadensis Miller). BiAloe® is the highest quality, most bio-available (in Blue), most immunomodulatory (in Red) and the highest total (in Green)

BIAloe® Is Organic Aloe vera Inner Leaf Freeze Dried Powder.
HIGHEST Total Acemannan – On Average 18%
HIGHEST Immunomodulatory Acemannan < 400 KDa
HIGHEST Bio-available Acemannan < 50 KDa
FULL SPECTRUM of Molecular Weight Polysaccharides
HIGHEST Polysaccharide Content – On Average 20%



	BiAloe	M	D	A
Total Acemannan	17.4%	12.7%	12.1%	9.9%
Fraction < 400 KDa	15.1%	4.1%	1.2%	6.3%
Fraction < 50 KDa	7.1%	1.6%	0.3%	1.8%

Polysaccharides between 400 and 5 KDa exhibit the most potent immunomodulatory activity.

Im SA, Oh ST, Song S, et al. Identification of optimal molecular size of modified Aloe polysaccharides with maximum immunomodulatory activity. International Immunopharmacology. 2005;5(2):271-279.

Materials and Methods: Samples A, B, D & M were ranked based upon their respective Total Acemannan. Numerous "other" samples were tested; however, they did not qualify for inclusion in the table since they lacked the full range of immunomodulatory Acemannan. For each sample listed, the Total Acemannan was calculated using the "o-acetyl method" then checked against the SEC (size exclusion chromatography) for accuracy using total polysaccharides as the marker. The SEC data was also used to determine the Fraction < 50 KDa and the Fraction < 400 KDa. Both tests were performed by different independent laboratories using blind samples.

Appendix 2

Material and Equipments Used for Preparation and Test

Materials and Equipments used in the preparation of HMC gel Appendix 4


Mixer (Kitchenaid model 5KPM50E)

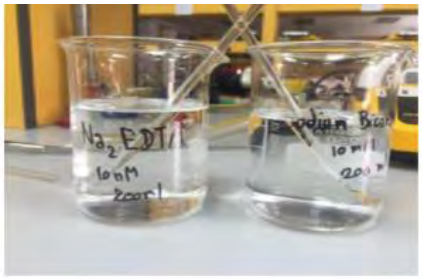

Vacuum Pump (GAST, model DOA-P504-BN)

PH-Meter and Phosphate Buffer



Franz Cell and Phosphate Buffer



UV-visible spectrophotometer (Shimazu, model UV 1800

Fillter Paper and Buffer Solution Filtered



Beeswax (B/No.515110, Namsiang CO., LTD)

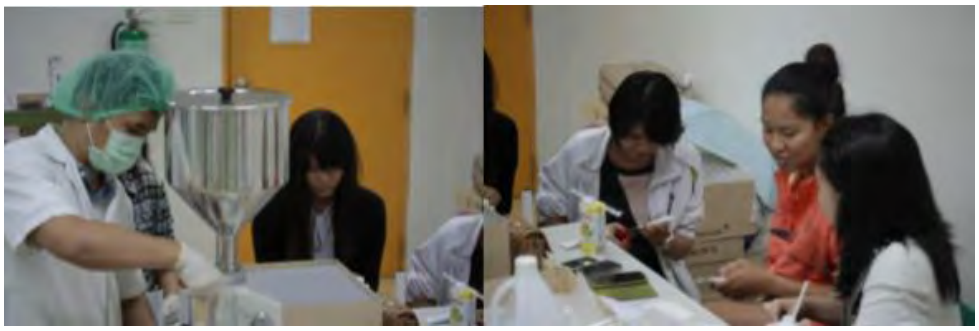
Carbopol 940 (B/No.0101521985, Namsiang CO.LTD)

Triethanolamine (B/No. D676EBLT05, Namsiang CO, LTD)

Glycerine (B/No. JOO1505081, Honghuat CO, LTD)

Di-Sodium hydrogen phosphate (B/No. 6M188277D, Carloerbareagents Potassium dihydrogen phosphate (B/No. V1L877228 Carlo-Erbareagents) Disodium EDTA (B/No. N052187E, Strongchemical CO., LTD) Sodium Chloride (B/No.0811292, Ajax Finechem PTY LTD)

Preparation and packagining of finished products, preparation to dispatch.



Appendix 3

Formulation of Test Product

(Extemporaneous Preparation)

Formulation of herbal medicine Combination

1. Cabophol 940 25 gm
2. Acetylsalicylic Acid 20 gm
   (2%)
3. สารสกัดว่านหางจรเข้ ที่มี Acemannan 17% eq.Acemannan 10 gm (1%)
4. สารสกัดใบบัวบกที่มี Asiaticoside 40% eq Asiaticoside 20 gm
   (2%)
5. Glycerine 10 ml
6. Dimethyl Isosorbide 30 ml
7. Methyl/Propyl Paraben 0.1% in Propyline Glycol 10ml
8. Triethanolamine 70 ml
9. Camphor 20 gm
10. Beeswax 10 gm
11. Isopropyl Alcohol 500 ml

Water qs. 1,000 ml

Formulation of Controlled Gel

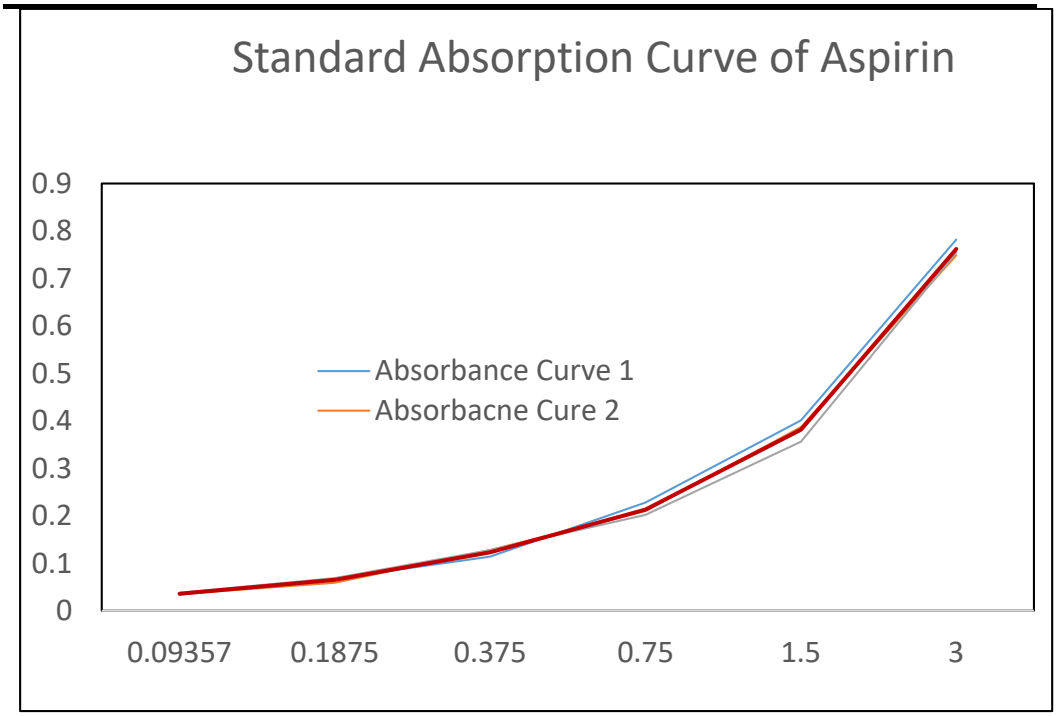
1. Cabophol 940 25 gm
2. Acetylsalicylic Acid 20 gm
   (2%)
3. Glycerine 10 ml
4. Dimethyl Isosorbide 30 ml
5. Methyl/Propyl Paraben 0.1% in Propyline Glycol 10ml
6. Triethanolamine 70 ml
7. Camphor 20 gm
8. Beeswax 10 gm
9. Isopropyl Alcohol 500 ml

Water qs. 1,000 ml

Appendix 4

Table – Aspirin and UV Visible Curve

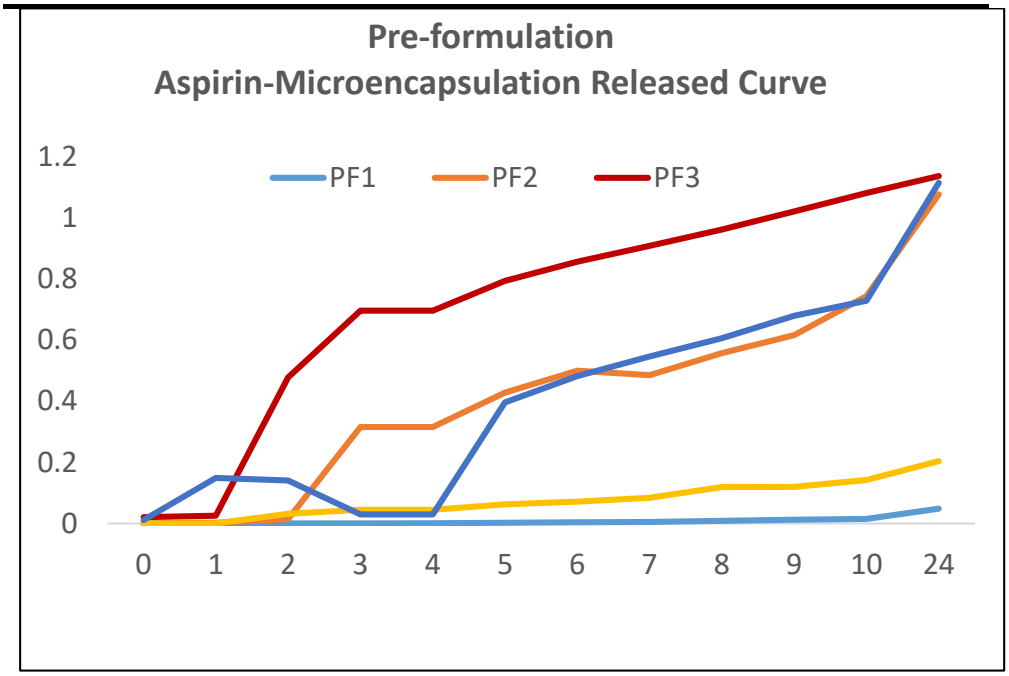
Concentration	Absorbacne at 302 nm				
(mg/ml)	1	2	3	Average	SD
0.09375	0.034	0.035	0.038	0.0357	0.0021
0.18750	0.068	0.059	0.069	0.0653	0.0255
0.37500	0.114	0.128	0.128	0.1233	0.0201
0.75000	0.228	0.210	0.202	0.2133	0.0132
1.50000	0.401	0.387	0.356	0.3813	0.0232
3.00000	0.782	0.749	0.755	0.7620	0.0176



Appendix 5

Aspirin Release – Franz Cell Analysis

Cumulative percent aspirin released (mg/mm2)					
Time (hr)	F1	F2	F3	F4	F5
0	0.0000.	0.0000	0.0207	0.0000	0.0111
1	0.0000	0.0021	0.0252	0.0000	0.1486
2	0.0000	0.0143	0.4776	0.0317	0.1402
3	0.0004	0.3151	0.6954	0.0445	0.2960
4	0.0007	0.3151	0.6954	0.0445	0.2960
5	0.0022	0.4277	0.7928	0.0625	0.3955
6	0.0037	0.4996	0.8556	0.0715	0.4817
7	0.0050	0.4848	0.9073	0.0840	0.5459
8	0.0084	0.5567	0.9611	0.1191	0.6057
9	0.0120	0.6156	1.0202	0.1196	0.6790
10	0.0148	0.7434	1.0803	0.1418	0.7279
24	0.0484	1.0758	1.1359	0.2037	1.1138



Appendix 6

Microbial Analysis

ผลการวิเคราะห์จุลินทรีย์ใน Chronic venous disease gel ชุดที่ 102

1. Total aerobic microbial count (TAMC) 0 CFU/g
2. Total yeast and mold count (TYMC) 0 CFU/g
3. Test for specified microorganisms

   ตรวจไม่พบ *Staphylococcus aureus* และ *Pseudomonas aeroginosa* ในตัวอย่าง 1 g

Acceptance criteria for microbiological quality of non-sterile dosage form (USP 38)

Preparation for cutaneous use

1. TAMC = 10^2 CFU/g ⟶ maximum acceptable count = 200 CFU/g
2. TYMC = 10^1 CFU/g ⟶ maximum acceptable count = 20 CFU/g
3. Absence of *Staphylococcus aureus* and *Pseudomonas aeroginosa* in 1g or 1 ml of sample

รศ. ดร. ภญ. นงลักษณ์ ศรีอุบลมาศ
ผู้วิเคราะห์
21 มิ.ย. 2559

Appendix 7

Patient Informed Consent

หนังสือให้ความยินยอมเข้าร่วมในโครงการวิจัย

วันที่...../...../.....

ข้าพเจ้า.....อายุ.....ปีอยู่บ้านเลขที่..... ถนน.....หมู่ที่ .....แขวง/ตำบล.....เขต/.....อำเภอ..... จังหวัด.....รหัสไปรษณีย์.....โทรศัพท์ .....

ขอทำหนังสือนี้ให้ไว้ต่อหัวหน้าโครงการวิจัยเพื่อเป็นหลักฐานแสดงว่า

ข้อ 1. ข้าพเจ้าได้รับทราบ**โครงการยุทธศาสตร์การวิจัยเชิงลึกคลัสเตอร์สังคมผู้สูงวัยของจุฬาลงกรณ์มหาวิทยาลัย เรื่อง** ประสิทธิภาพและความปลอดภัยของตำรับยาสมุนไพรสกัดผสมกรดอะเซทิลซาลิไซลิก,สมุนไพรสกัดใบบัวบก,อะซีแมนแนนสกัดจากว่านหางจระเข้ชนิดเจลทาภายนอก เพื่อรักษาอาการสาเหตุจากโรคหลอดเลือดดำหย่อนสมรรถภาพในคนไทย : การศึกษาแบบสุ่มเปรียบเทียบกับยาหลอกชนิดผู้รักษาและผู้เข้ารับการรักษาไม่ทราบชนิดของยา มี หัวหน้าโครงการวิจัยคือ นายแพทย์ พิชัย พงษ์มั่นจิตผศ, เภสัชกร อนันต์ อุดมพรประภา, ดร. เนาวรัตน์ กาญจนาคาร, ศาสตราจารย์ นพ สุรศักดิ์ ฐาณีพานิชกุล, , ผู้ช่วยศาสตราจารย์ เภสัชกร วิเชียร ธานินทร์ธราธาร และคณะ ฯ

ข้อ 2. ข้าพเจ้ายินยอมเข้าร่วมโครงการวิจัยนี้ด้วยความสมัครใจ โดยมิได้มีการบังคับหลอกลวงแต่ประการใด และจะให้ความร่วมมือในการวิจัยทุกประการ

ข้อ 3. ข้าพเจ้าได้รับการอธิบายจากผู้วิจัยเกี่ยวกับวัตถุประสงค์ของการวิจัย วิธีการวิจัย และการวิจัยนี้ไม่มีการจ่ายค่าตอบแทน แต่ค่าใช้จ่ายที่จะใช้ในการรักษาโรคและอาการจากหลอดเลือดดำหย่อนสมรรถภาพของท่าน
เป็นไปตามมาตรฐานการรักษาของโรงพยาบาล และท่านจะได้รับยาที่นำมาศึกษาเพื่อดูผลการรักษาแบบไม่มีค่าใช้จ่ายใดๆ และจะได้รับการดูแลตามมาตรฐานปกติแบบไม่คิดค่าใช้จ่ายจากยาที่นำมารักษานี้ เมื่อได้เข้ารับการประเมินติดตามผลการรักษาอย่างต่อเนื่องทั้งหมดหก (6)ครั้ง

การเจาะเลือด นับจากการเข้าร่วมโครงการ ท่านอาจจำเป็นจะต้องได้รับการเจาะเลือดสองครั้ง ครั้งแรกเมื่อเริ่มการศึกษาและครั้งที่สองเมื่อสิ้นสุดการศึกษาในเดือนที่สาม (ปริมาณเลือดที่ใช้ครั้งละ 8-10 CC)

การติดตามผลการรักษา แพทย์ที่ทำการรักษาท่านจะนัดท่าน เพื่อตรวจประเมินรวมทั้งหมดหกครั้งรวมทั้งครั้งแรก เมื่อท่านตอบรับเข้าร่วมการศึกษา(ครั้งที่1) หลังจากนั้นแพทย์จะนัดท่าน มาเมื่อครบหนึ่งสัปดาห์(ครั้งที่

2) เมื่อครบสัปดาห์ที่สอง (ครั้งที่ 3) เมื่อครบสัปดาห์ที่สี่ (ครั้งที่ 4) เมื่อครบสัปดาห์ที่แปด (ครั้งที่ 5) และเมื่อครบ
สัปดาห์ที่สิบสอง (ครั้งที่ 6) เมื่อสิ้นสุดการศึกษา

โดยท่านได้อ่านข้อความที่มีรายละเอียดอยู่ในเอกสารชี้แจงผู้เข้าร่วมโครงการวิจัยโดยตลอด อีกทั้งยังได้รับคำอธิบาย และตอบข้อสงสัยจากหัวหน้าโครงการวิจัยเป็นที่เรียบร้อยแล้ว และตกลงรับผิดชอบตาม คำรับรองใน **ข้อ** 5 ทุกประการ

ข้อ 4. ข้าพเจ้าได้รับรองจากผู้วิจัยว่า จะเก็บข้อมูลส่วนตัวของข้าพเจ้าเป็นความลับเปิดเผยเฉพาะผลสรุปกาะวิจัยเท่านั้น

ข้อ 5. ข้าพเจ้าได้รับทราบจากผู้วิจัยแล้วว่า หากมีอันตรายใดๆอันเกิดขึ้นกับการวิจัยดังกล่าว ข้าพเจ้าจะได้รับการรักษา พยาบาลจากคณะผู้วิจัยโดยไม่คิดค่าใช้จ่ายตามมาตรฐานโรงพยาบาลเชียงรายประชานุเคราะห์

ข้อ 6. ข้าพเจ้าได้รับทราบแล้วว่าข้าพเจ้ามีสิทธิ์จะบอกเลิกการร่วมโครงการวิจัยนี้ และการบอกเลิกโครงการวิจัยนี้จะไม่มีผลกระทบต่อการดูแลรักษาโรคที่ข้าพเจ้าจะพึงได้รับต่อไป

ข้อ 7. หากข้าพเจ้ามีข้อข้องใจใดเกี่ยวกับขั้นตอนการวิจัย หรือหากเกิดผลข้างเคียงที่ไม่พึงประสงค์จากการวิจัยสามารถติดต่อกับ หัวหน้าโครงการวิจัย และ/หรือ เภสัชกร คมกริช ศรีไสว ในฐานะที่ปรึกษาร่วมวิจัยได้ตามที่อยู่และเบอร์โทรศัพท์ข้างล่างนี้

ข้อ 8. กรณีท่านมีข้อสงสัยเกี่ยวกับสิทธิของท่านในการเข้าร่วมโครงการวิจัย กรุณาติดต่อได้ที่ สำนักงเลขานุการคณะกรรมการพิจารณาด้านจริยธรรมในการศึกษาวิจัยทางชีวเวชศาสตร์ โรงพยาบาลเชียงรายประชาเคราะห์ โทร 053 711300 ต่อ 2145

ข้าพเจ้าได้อ่านและเข้าใจตามหนังสือนี้โดยตลอดแล้วเห็นว่าถูกต้องตามเจตนาของข้าพเจ้าจึงได้ลงลายมือชื่อเป็นสำคัญพร้อมกับหัวหน้าโครงการวิจัยและต่อหน้าพยาน

ลงชื่อ..... ลงชื่อ .....

(.....)

(.....)

ผู้ยินยอม/ผู้แทนโดยชอบธรรม ผู้ให้ข้อมูลและขอความยินยอม/หัวหน้าโครงการวิจัย

ลงชื่อ.....พยาน ลงชื่อ .....พยาน

(.....)

(.....)

ในกรณีผู้ร่วมวิจัยอ่านหนังสือไม่ออก ผู้ที่อ่านข้อความทั้งหมดแทนผู้เข้าทำการวิจัยคือ

.....

.....

.....

จึงได้ลงลายมือชื่อไว้เป็นพยาน

ลงชื่อ.....พยาน ลงชื่อ

.....พยาน

(.....)

(.....)

หมายเหตุ

1. กรณีผู้ยินยอมตนให้ทำการวิจัย ไม่สามารถอ่านหนังสือได้ให้ผู้วิจัยอ่านข้อความในหนังสือให้ความยินยอมนี้ให้แก่ผู้ยินยอมให้ทำวิจัยฟังจนเข้าใจ และให้ผู้ยินยอมตนให้ทำวิจัย ลงนามหรือพิมพ์ลายนิ้วหัวแม่มือรับทราบในการให้ความยินยอมดังกล่าวด้วย

คำแนะนำการใช้ยา

อาการข้างเคียงที่อาจเกิดจากการทายาเจลที่ศึกษา

1. ท่านอาจมีอาการคันหรือระคายเคืองที่เท้าบริเวณที่ทายาภายหลังจากทายา อาการเหล่านี้จะเป็นเพียงอาการระคายเคืองภายนอกเพียงเล็กน้อย และสามารถหายได้เองภายใน 2-3 ชั่วโมง อย่างไรก็ตามหากอาการระคายเคืองนี้ยังคงอยู่และท่านไม่สามารถทนทานได้ ให้ท่านล้างออกด้วยน้ำสะอาด แล้วเช็ดให้แห้งจะทำให้อาการระคายเคืองหายไป หากอาการยังปรากฏอยู่ภายหลลังจากหยุดทายาและล้างออกแล้ว ให้ติดต่อโดยตรงหรือปรึกษาที่ผู้วิจัยและเภสัชกร ที่หมายเลข 089-129-6285 / 081-671-4057

2. ท่านอาจมีความรู้สึกเหนียวเหนอะหนะที่เท้าบริเวณที่ทายา อาการเหล่านี้จะเป็นเพียงอาการภายนอกเพียงเล็กน้อยและสามารถหายได้เองเมื่อเจลแห้งตัวลงในเวลาต่อมา

3. ท่านจำเป็นต้องทายาเจลวันละสองครั้งเช้าและเย็นก่อนนอนให้ทั่วๆเท้า

4. หากท่านมีอาการผด ผื่นคัน บริเวณเท้า ที่อาจเกิดจากการแพ้ยาหรือสารผสมในยาเจล หรืออาการเท้าบวมภายหลังจากทายาให้ใช้น้ำสะอาดล้างออก หยุดใช้ยาและรายงานให้แพทย์หรือผู้วิจัยทราบ

คำแนะนำในการปฏิบัติตนสำหรับผู้ป่วยที่มีอาการเจ็บปวดเท้าจากสาเหตุของโรคหลอดเลือดดำหย่อนสมรรถภาพทั่วไป

การรับประทานยา

1. ห้ามรับประทานยาแผนโบราณ หรือยาแผนปัจจุบันอื่นๆ ที่อาจกัดกระเพาะและมีผลทำให้อาการปวดท้อง และ อาการอื่นๆที่ท่านกำลังได้รับการรักษารุนแรงขึ้น ยกเว้น ได้รับการปรึกษากับแพทย์ผู้รักษาและมีความจำเป็นจริงๆ เฉพาะกรณี หากมีข้อสงสัยและจำเป็นต้องได้รับการรักษาให้แจ้งและปรึกษาแพทย์และเภสัชกร
2. ขณะนี้ท่านกำลังได้รับการรักษาและอยู่ในงานศึกษาวิจัย การรับประทานยาใดๆระหว่างการศึกษาควรได้รับการปรึกษากับแพทย์ผู้รักษาก่อน

การทายาเจล

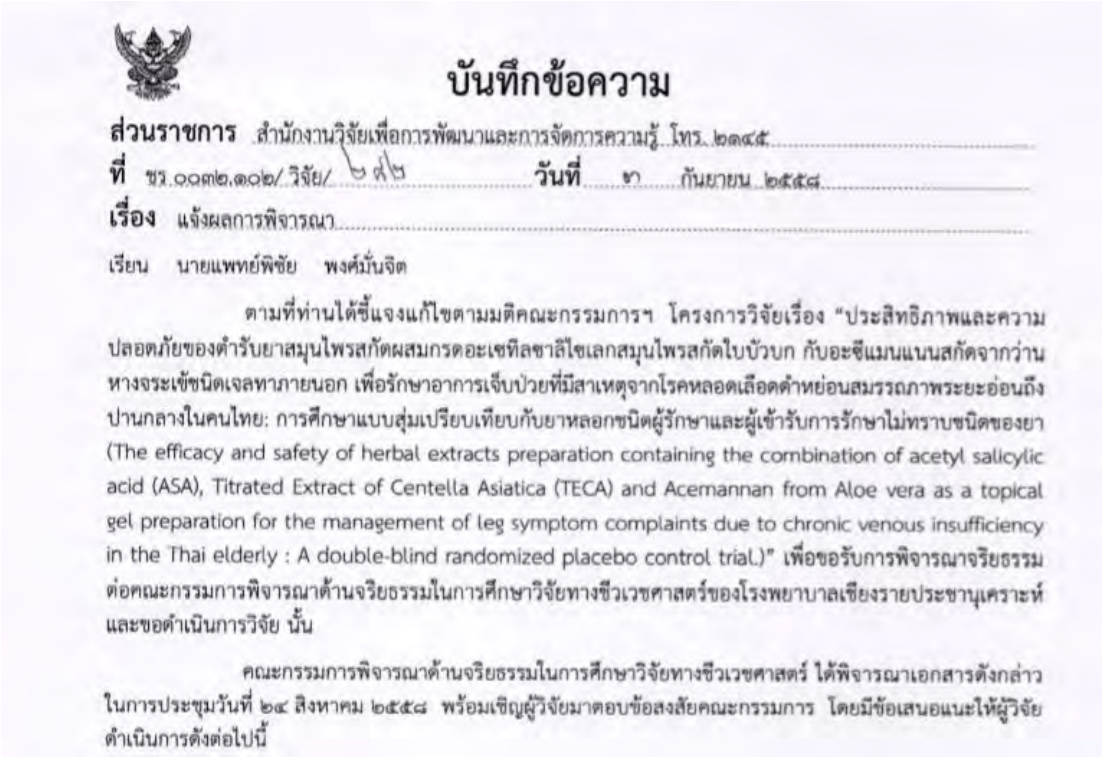
1. ยาเจลนี้เป็นยาสำหรับใช้ภายนอกเท่านั้น **ห้ามรับประทานโดยเด็ดขาด**
2. ขณะนี้ท่านกำลังได้รับการรักษาและอยู่ในงานศึกษาวิจัย การทายาเจล **ควรทาให้สม่ำเสมอทั่วเท้าใน ปริมาณตาม ที่แพทย์สั่งเท่านั้น โดยทาเพียงวันละสองครั้ง เช้าและก่อนนอนเท่านั้น**
3. ภายหลังทายา ห้ามขัดถู แ**ต่ให้ทายาเจลให้บาง ๆพอประมาณเริ่มแรกจะมีความเหนียวเหนอะหนะ ทิ้งไว้ประมาณ 10-20 นาที ยาเจลจะเปลี่ยนเป็นแผ่นฟิลม์บางเรียบผิวลดอาการเหนียวเหนอะหนะ**
4. เก็บรักษายาในหลอดไว้ที่อุณหภูมิระหว่าง 15-25 องศาเซลเซียส หรืออาจเก็บในตู้เย็น

การดูแลสุขภาพและการพักผ่อน

1. ไม่ควรยืนนิ่งอยู่กับที่เป็นเวลานานๆ ถ้าจำเป็นควรเปลี่ยนอริยาบทบ่อยๆ
2. หลีกเลี่ยงไม่ให้ขาสัมผัสกับความร้อน เช่น อาบน้ำที่ร้อนเกินไป ยืนบนพื้นร้อนๆ อาบแดดนานๆ
3. **ควรสวมรองเท้าที่สูงไม่เกิน** 5 **ซม**.
4. ในกรณีที่ต้องยืนนาน ๆ ควรสวมถุงน่องที่ช่วยพยุง และกระชับกล้ามเนื้อขา ซึ่งมีแรงบีบรีดไม่น้อยกว่า 30 มิลลิเมตรปรอท และควรสวมตั้งแต่เท้าจนถึงเหนือเข่า
5. ออกกำลังกายที่ช่วยให้กล้ามเนื้อ และหลอดเลือดแข็งแรง เช่น ว่ายน้ำ วิ่ง ขี่จักรยาน แต่ควรหลีกเลี่ยงกีฬาที่รุนแรงเกินไป เช่น กระโดดสูง กระแทกเท้า ควรออกกำลังกายสม่ำเสมอ และละเว้นการออกกำลังกายแบบหักโหม หมั่นทำกิจกรรมที่สามารถลดความเครียด เช่น การทำกิจกรรมออกกำลังกายแบบแอโรบิกเดี่ยวๆ หรือเป็นกลุ่ม
6. ยกเท้าสูงประมาณ 45 องศา ขณะนอนพัก จนกระทั่งรู้สึก สบายขึ้น จึงนอนต่อในท่าปกติ
7. ไม่ควรปล่อยให้น้ำหนักตัวเพิ่มมากเกินไป หมั่นดูแลระมัดระวังควบคุมน้ำหนักตัวให้คงที่ หรือลดน้ำหนักสำหรับผู้ที่มีน้ำหนักมาก ภายใต้คำแนะนำของแพทย์
8. การพักผ่อนให้เพียงพอ และพยายามเข้านอนและตื่นนอนให้ตรงเวลาสม่ำเสมอ

Appendix 8

Hospital Ethical Review Certificate



บันทึกข้อความ

ส่วนราชการ สำนักงานวิจัยเพื่อการพัฒนาและการจัดการความรู้ โทร. ๒๑๔๕

ที่ ชร.๐๐๓๒.๑๐๒/ วิจัย/ ๒๙๒ **วันที่** ๓ กันยายน ๒๕๕๘

เรื่อง แจ้งผลการพิจารณา

เรียน นายแพทย์พิชัย พงศ์มั่นจิต

ตามที่ท่านได้ชี้แจงแก้ไขตามมติคณะกรรมการฯ โครงการวิจัยเรื่อง "ประสิทธิภาพและความปลอดภัยของตำรับยาสมุนไพรสกัดผสมกรดอะเซทิลซาลิไซเลกสมุนไพรสกัดใบบัวบก กับอะซีแมนแนนสกัดจากว่านหางจระเข้ชนิดเจลทาภายนอก เพื่อรักษาอาการเจ็บป่วยที่มีสาเหตุจากโรคหลอดเลือดดำหย่อนสมรรถภาพระยะอ่อนถึงปานกลางในคนไทย: การศึกษาแบบสุ่มเปรียบเทียบกับยาหลอกชนิดผู้รักษาและผู้เข้ารับการรักษาไม่ทราบชนิดของยา (The efficacy and safety of herbal extracts preparation containing the combination of acetyl salicylic acid (ASA), Titrated Extract of Centella Asiatica (TECA) and Acemannan from Aloe vera as a topical gel preparation for the management of leg symptom complaints due to chronic venous insufficiency in the Thai elderly : A double-blind randomized placebo control trial.)" เพื่อขอรับการพิจารณาจริยธรรมต่อคณะกรรมการพิจารณาด้านจริยธรรมในการศึกษาวิจัยทางชีวเวชศาสตร์ของโรงพยาบาลเชียงรายประชานุเคราะห์ และขอดำเนินการวิจัย นั้น

คณะกรรมการพิจารณาด้านจริยธรรมในการศึกษาวิจัยทางชีวเวชศาสตร์ ได้พิจารณาเอกสารดังกล่าวในการประชุมวันที่ ๒๔ สิงหาคม ๒๕๕๘ พร้อมเชิญผู้วิจัยมาตอบข้อสงสัยคณะกรรมการ โดยมีข้อเสนอแนะให้ผู้วิจัยดำเนินการดังต่อไปนี้

๑. แสดงเอกสารเกี่ยวกับการวิจัยด้วยาแต่ละตัว ที่บ่งชี้ว่าเป็นการวิจัยระยะ ๓ และ เอกสารการทดลองความคงตัวของยา

๒. ทำบันทึกข้อความปรึกษา กลุ่มงานเภสัชกรรมโรงพยาบาลเชียงรายฯ เรื่อง ระบบการบริหารยาโครงการวิจัยของโรงพยาบาล เพื่อให้การจัดเก็บและควบคุมการกระจายยาวิจัยมีประสิทธิภาพ โดยแบบโครงร่างงานวิจัย ข้อมูลยา (เช่น สูตรยา การคงตัวของยา การผ่านการรับรองยา เป็นต้น) และคำแนะนำเรื่องข้อห้าม /การแพ้ยา / ผลข้างเคียงของยา/ การจัดการ และ เอกสารหลักฐานอื่น ๆ ที่เกี่ยวข้อง

๓. ทบทวน และแก้ไข เอกสารชี้แจง และแสดงความยินยอม เกี่ยวกับ

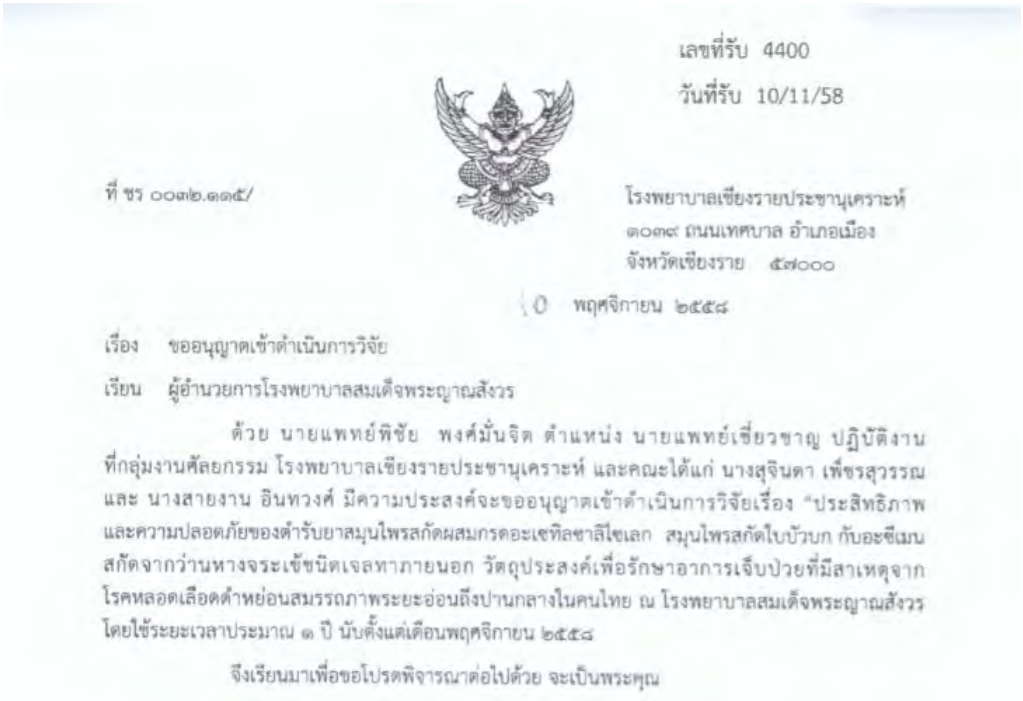
- ข้อมูลการดำเนินการวิจัยที่ชัดเจนแต่ละขั้นตอน ระยะเวลาที่ใช้ ความถี่ในการนัดหมาย ปริมาณเลือดที่เจาะ การตอบแบบสอบถาม มีการการประเมินต่างๆ ที่ดำเนินการในการวิจัย
- คำแนะนำเรื่องข้อห้าม /การแพ้ยา / ผลข้างเคียงของยา / การจัดการ กรณีเกิดเหตุการณ์ไม่พึงประสงค์ และผู้ติดต่อ
- ผู้วิจัยชี้แจงว่าไม่มีค่าตอบแทนการเข้าร่วมโครงการวิจัย การชดเชยรายได้ หรือค่าตอบแทนความพิการกรณีที่อาจเกิดขึ้นจากการวิจัย แต่ในเอกสารแสดงความยินยอมข้อ ๓ และ ๕ ยังมีการระบุเรื่องค่าตอบแทน ดังนั้น ขอให้แก้ไขให้ชัดเจน

เมื่อดำเนินการแก้ไขเป็นลายลักษณ์อักษรแล้ว ส่งมาให้คณะกรรมการพิจารณาอีกครั้ง

จึงเรียนมาเพื่อทราบและพิจารณาดำเนินการ

(แพทย์หญิงรวิวรรณ หาญสุทธิวรกุล)
ประธานคณะกรรมการพิจารณาด้านจริยธรรม
ในการศึกษาวิจัยทางชีวเวชศาสตร์

หมายเหตุ: คณะกรรมการฯ ได้ดำเนินการภายใต้หลัก ICH-GCP และด้านจริยธรรมการวิจัย
โดยกรรมการผู้เกี่ยวข้อง ไม่มีส่วนร่วมในการพิจารณาโครงการวิจัย



เลขที่รับ 4400
วันที่รับ 10/11/58

ที่ ชร ๐๐๓๒.๑๑๕/

โรงพยาบาลเชียงรายประชานุเคราะห์
๑๐๓๙ ถนนเทศบาล อำเภอเมือง
จังหวัดเชียงราย ๕๗๐๐๐

๑๐ พฤศจิกายน ๒๕๕๘

เรื่อง ขออนุญาตเข้าดำเนินการวิจัย

เรียน ผู้อำนวยการโรงพยาบาลสมเด็จพระญาณสังวร

ด้วย นายแพทย์พิชัย พงศ์มั่นจิต ตำแหน่ง นายแพทย์เชี่ยวชาญ ปฏิบัติงานที่กลุ่มงานศัลยกรรม โรงพยาบาลเชียงรายประชานุเคราะห์ และคณะได้แก่ นางสุจินดา เพ็ชรสุวรรณ และ นางสายงาน อินทวงศ์ มีความประสงค์จะขออนุญาตเข้าดำเนินการวิจัยเรื่อง "ประสิทธิภาพและความปลอดภัยของตำรับยาสมุนไพรสกัดผสมกรดอะเซทิลซาลิไซเลท สมุนไพรสกัดใบบัวบก กับอะซีเมนสกัดจากว่านหางจระเข้ชนิดเจลทาภายนอก วัตถุประสงค์เพื่อรักษาอาการเจ็บป่วยที่มีสาเหตุจากโรคหลอดเลือดดำหย่อนสมรรถภาพระยะอ่อนถึงปานกลางในคนไทย ณ โรงพยาบาลสมเด็จพระญาณสังวร โดยใช้ระยะเวลาประมาณ ๑ ปี นับตั้งแต่เดือนพฤศจิกายน ๒๕๕๘

จึงเรียนมาเพื่อขอโปรดพิจารณาต่อไปด้วย จะเป็นพระคุณ

ขอแสดงความนับถือ

(นายแพทย์พิชัย พงศ์มั่นจิต)
นายแพทย์เชี่ยวชาญ ด้านเวชกรรม สาขาศัลยกรรม
หัวหน้าโครงการวิจัยฯ

เรียน ผอก.รพ.สมเด็จพระญาณสังวร
เห็นควรอนุมัติ

(นางสาวโมทัพนันธ์ พนิสยาคา)
นักจัดการงานทั่วไป ชำนาญการ
10 พฤศจิกายน 2558

อนุมัติ

(นายชัชวัฒน์ ไชยคำรัง)
ผอก.รพ.สมเด็จพระญาณสังวร
10 พฤศจิกายน 2558

กลุ่มงานศัลยกรรม
โทร. ๐ ๕๓๗๑ ๑๓๐๐ ต่อ ๑๓๑๙
โทรสาร ๐ ๕๓๗๑ ๓๐๔๔

ทราบ แจ้งคุณสายงาน คุณสุจินดา

นาง อรุณศรี หงเจริญ
11 พฤศจิกายน 2558

ทราบ
11 พฤศจิกายน 2558

ทราบ

11 พฤศจิกายน 2558

Appendix 9

Physician Rated Symptoms Perception Score (PRSPS)

Symptos Assessed	Severe-5	Frequent-4	Sometime-3	Occasionaly-2	Not at all-1
Heavy Leg					
Pain in the Leg					
Sensation of Leg Itching					
Night Cramp					
Itching Leg					
Sensation of Burning					
Sensation of Pin/Needle in Leg					
Superficial Varicose Veins					
Presence of Spider Veins					
Remark. Severe= Every day, Frequent= Every other days, Sometime= Once a week					

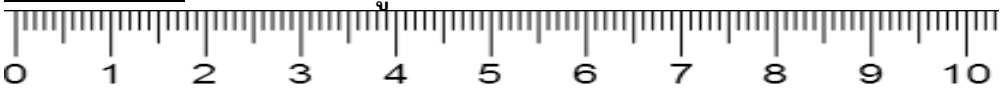
Notes Final score computation employed a transformed percentage scores from absent (0) to severe (100) and a standardized score.

Appendix 10

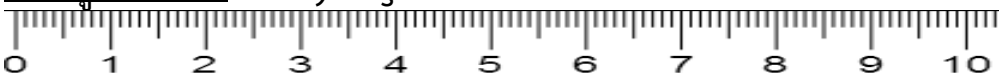
Patient Self-Rated Symptoms Scale (PSSS)

<u>แบบประเมินอาการโดยผู้ป่วย</u> : ความรู้สึกต่ออาการเจ็บป่วยของข้าพเจ้า

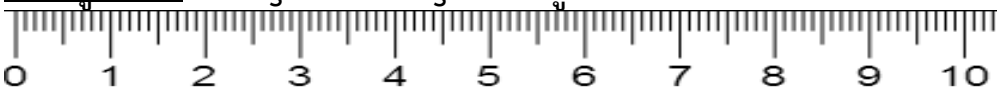
<u>อาการปวดขา</u> Pain : ความรู้สึกเจ็บปวดขา



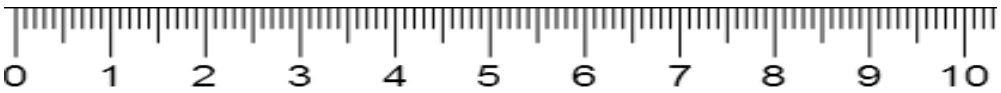
<u>ความรู้สึกขาหนัก</u> Heavy Leg:



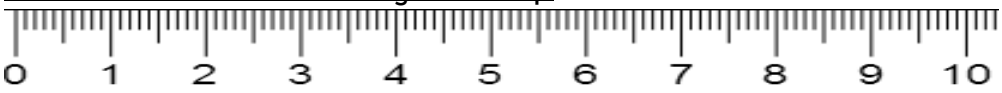
<u>ความรู้สึกคัน</u> Itching in the Leg : ความรู้สึกว่ามีอาการคัน



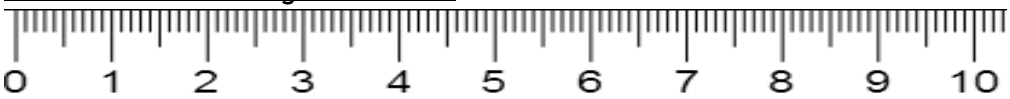
<u>อาการคล้ายเข็มแทง</u> Needle or Pin : ความรู้สึกคล้ายๆกับมีเข็มแทงภายใน



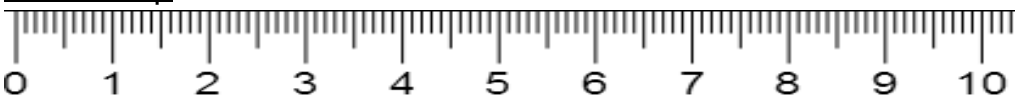
<u>อาการตะคริวเวลากลางคืน Night Cramp</u>



<u>อาการขาบวมน้ำ Leg Edema Cr</u>



<u>อาการอื่น ๆ 1:</u>.....



Notes

Each of categories of VAS measured in length (as centimeter) and the final total score combined employed a transformed percentage scores from absent (0) to severe (100).

Appendix 11

Venous Clinical Severity Score (VCSS)

VCSS	Severe -3	Moderate-2	Mild-1	Absent-0
Pain				
Varicose Vein				

Pain ***Absent*** *= None,* ***Mild*** *= Occasionnal not restricting activity or requiring analgesics,* ***Moderate*** *= Daily, moderate activity limitation requiring analgesics,* ***Severe*** *= Daily severe, limiting activity or requiring regular analgesics.*

Varicose Vein ***Absent*** *= None,* ***Mild*** *= Few Scatterred, Branch Varicose Vein,* ***Moderate*** *= Multiple, greater saphenous varios Vin ontinu to calf or thigh,* ***Severe*** *= Extensiv thigh an alf or gratr saphenous and lower saphenous extnsion*

VCSS	Severe-3	Moderate-2	Mild-1	Absent-0
Venous Edema				
Skin Pigmentation				

Venous Edema ***Absent*** *= None,* ***Mild*** *= Eveninig ankle edema only,* ***Moderate*** *= Afternoon edema above amkle,* ***Severe*** *= Morning edema above ankle requiring changes & elevation.*

Skin Pigmentation ***Absent*** *= None or focal low intensity (Tan),* ***Mild*** *= Diffuse but limited in area andold (Tan),* ***Moderate*** *= Diffuse over most of gaiter distribution,lower third or recent pigmentation (purple)* ***Severe*** *= Wider distribution, above or lower third or recent pigmentation.*

Notes Final score computation employed a transformed percentage scores from absent (0) to severe (100) and a standardized score.

Appendix 11 Venous Clinical Severity Score (VCSS) - continue

VCSS	Severe-3	Moderate-2	Mild-1	Absent-0
Inflammation				
Induration				

Inflammation ***Absent*** *= None,* ***Mild*** *= Mild Cellulitis and limited marginal area around ulcer,* ***Moderate*** *= Moderate cellulitis involved most of gaiter area,* ***Severe*** *= Severe cellulitis or significant venous eczema.*

Induration ***Absent****=None,* ***Mild****=focal circummalleolar (<5cm),* ***Moderat****e = medial or lateral or less than lower third of leg,* ***Severe*** *= Entire lower third of leg or more*

VCSS	Severe-3	Moderate-2	Mild-1	Absent-0
No. of Active Ulcers				
Acvtive ulceration duartion				

No of active Ulcer ***Absent*** *=0,* ***Mild*** *=1,* ***Moderate*** *= 2,* ***Severe*** *= > 2*

Active Ulceration duration ***Absent****=None,* ***Mild****=< 3 months,* ***Moderat****e => 3 month to < 1 year,* ***Severe*** *= not heal mor than 1 year*

Notes Final score computation employed a transformed percentage scores from absent (0) to severe (100) and a standardized score.

Appendix 12

Medical Outcomes Studies 12-Item

Short-Form Health Survey (MOS SF12)

คำถาม	ดีเยี่ยม-1	ดีมาก-2	ดี-3	พอใช้-4	ไม่ดี-5
1. โดยทั่วๆไปท่านสามารถพูดได้ว่าสุขภาพของท่านเป็นอย่างไร					

เรื่องต่อไปนี้เป็นเรื่องเกี่ยวกับกิจกรรมที่ท่านทำในแต่ละวัน ท่านคิดว่าสุขภาพของท่านเป็นปัญหาหรืออุปสรรคในการทำกิจกรรมของท่านหรือไม่ ถ้าใช่ มากน้อยแค่ไหน			
	ใช่เป็นปัญหาอุปสรรคอย่างมาก-1	ใช่,เป็นปัญหาอุปสรรค เพียงเล็กน้อย-2	ไม่เป็นปัญหาอุปสรรคเลย-3
2. กิจกรรมที่ใช้กำลังปานกลาง เช่น การยกโต๊ะ การทำความสะอาดปัดกวาดเช็ดถูบ้าน หรือ หิ้วของ กลับจากตลาด			
3. การเดินขึ้นตึก สอง ถึง สามชั้นหรือขึ้นเนิน			

ในช่วงหนึ่งเดือนที่ผ่านมาท่านเคยมีปัญหาในเรื่องต่อไปนี้กับการงานของท่านหรือกิจกรรมที่ทำเป็นประจำทุกวัน เนื่องมาจากสุขภาพของท่านใช่หรือไม่		
	ใช่-1	ไม่ใช่-2
4. ทำงานได้ปริมาณน้อยลงกว่าที่ต้องการ		
5. ไม่สามารถทำงานได้ทุกอย่างตามที่ตั้งใจไว้ ต้องเลือกทำบางอย่างเท่านั้น		

Appendix 12 Medical Outcomes Studies 12-Item Short-Form Health Survey (MOS SF12)

ในช่วงหนึ่งเดือนที่ผ่านมา เคยมีปัญหาในเรื่องต่อไปนี้ กับงานของท่านหรือกิจกรรมที่ทำเป็นประจำทุกวัน เนื่องมาจากปัญหาด้านอารมณ์ของท่านหรือไม่ (เช่น ความรู้สึกซึมเศร้าหรือวิตกกังวล)			
		ใช่-1	ไม่ใช่-2
6. ฉันทำงานได้ปริมาณน้อยลงกว่าที่ต้องการ			
7. ฉันทำงาน หรือทำกิจกรรมอื่นๆโดยปราศจากความระมัดระวัง สับเพร่า เลินเล่ออย่าง ไม่ดีที่เคย			

8. ในระหว่างหนึ่งเดือนที่ผ่านมา ปัญหาการเจ็บป่วยตามร่างกายทำให้ฉันไม่สามารถทำงานประจำวันได้ตามปกติ (งานในบ้านและนอกบ้าน) มากน้อยเพียงใด				
ไม่เลย	เล็กน้อย	ปานกลาง	ค่อนข้างมาก	มากที่สุด

คำถามต่อไปนี้เกี่ยวข้องกับความรู้สึกและสิ่งต่างๆ ที่เกิดขึ้นกับท่านในช่วงหนึ่งเดือนที่ผ่านมากรุณาเลือกข้อที่ตรงกับความรู้สึกของท่านมากที่สุดเพียงข้อเดียว						
	ตลอดเวลา-1	เกือบตลอดเวลา-2	ค่อนข้างบ่อย-3	บางครั้ง-4	นานๆ ครั้ง-5	ไม่เลย-6
9.ฉันรู้สึกใจสงบ ใจสบาย ใจนิ่ง มีสมาธิ						
10.ฉันรู้สึกแข็งแรง กระปรี้กระเปร่า สดชื่น						
11.ฉันรู้สึกเศร้า หดหู่						
12. ในช่วงหนึ่งเดือนที่ผ่านมาบ่อยแค่ไหนที่ท่านรู้สึกว่าปัญหาสุขภาพหรือปัญหาทางอารมณ์เป็นอุปสรรคขัดขวางการทำกิจกรรมสังคมของท่าน เช่นการไปเยี่ยมเพื่อน/ญาติสนิทเป็นต้น						

Appendix 13

Medical Outcomes Studies 14-Item

Chronic Venous Disease Short-Form Health Survey (MOS CIVIQ14)

ในสี่สัปดาห์ที่ผ่านมา ท่านมีอาการปวดที่ข้อเท้าหรือขา และความรุนแรงของอาการปวดเป็นอย่างไร	ไม่ปวด	ปวดเล็กน้อย	ปวดปานกลาง	ปวดมาก	ปวดรุนแรง
	1	2	3	4	5
ในสี่สัปดาห์ที่ผ่านมา ท่านมีความลำบากในการทำงานหรือใช้ชีวิตประจำวันแค่ไหน เนื่องจากปัญหาที่ขาหรือข้อเท้า	ไม่ลำบาก	ลำบากเล็กน้อย	ลำบากปานกลาง	ลำบากมาก	ลำบากรุนแรง
	1	2	3	4	5
ในสี่สัปดาห์ที่ผ่านมา ท่านนอนไม่หลับเนื่องจากปัญหาที่ขาหรือข้อเท้ามากแค่ไหน	ไม่เคย	น้อยครั้ง	บ่อย	บ่อยมาก	ทุกวัน
	1	2	3	4	5

	ไม่ลำบาก-1	ลำบากเล็กน้อย-2	ลำบากปานกลาง-3	ลำบากมาก-4	ไม่สามารถทำได้-5
ขึ้นบันไดหลายชั้น					
คุกเข่า นั่งคุกเข่า					
เดินเร็ว					
ออกจากบ้านช่วงเย็นไปงานเลี้ยง					
เล่นกีฬา ออกแรง					

ปัญหาที่ขาและข้อเท้าที่อาจมีผลต่ออารมณ์ ท่านรู้สึกอย่างไรต่อไปนี้ในช่วงสี่สัปดาห์ที่ผ่านมา

	ไม่เคย-1	เล็ก น้อย-2	ปานกลาง-3	มาก-4	มากที่สุด-5
ฉันรู้สึกหงุดหงิด เครียด					
ฉันรู้สึกว่าตนเองเป็นภาระ					
ฉันรู้สึกอายที่ให้คนอื่นเห็นขา					
ฉันรู้สึกโกรธ ฉุนเฉียวง่าย					
ฉันรู้สึกว่าตนเองพิการ					
ฉันไม่อยากออกไปข้างนอก					

Appendix 14

Summary Adverse Drug Reactions

Spontaneous Report of Adverse Drug Reactions (Suspected)
แบบรายงานอาการไม่พึงประสงค์จากยา **ชนิดรายงานโดยผู้ป่วย**

	อาการไม่พึงประสงค์ที่ท่านสงสัย โปรดระบุรายละเอียด	
	วัน เวลา ที่เกิดอาการ ระหว่างเวลาใด	**มีอาการพอทนได้ (1)** **ไม่มีอาการ (2)** **มีอาการหนักต้องหยุดยา (3)**
1.		
2.		
3.		
4.		
5.		
6.		
7.		
8.		
9.		
10.		
11.		
12.		
13.		

หมายเหตุ
รายงานอาการไม่พึงประสงค์นี้เป็นการบันทึกรายงานตามสมัครใจของผู้ป่วยประจำวันก่อนพบแพทย์เพื่อประเมิน ให้ระบุเป็นความรุนแรงตามหมายเลข

VITA

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PUBLICATION	1. Kiatsangorn T, Pono K, Suwannarat K, Kittiphinitnunta K, Udombhornprabha A. A One –Year Cost of Illness of Schizophrenia Patients in A Public Psychiatric Hospital with Implication of Direct Medical Cost and Antipsychotics. Accepted for as poster presentation for the ISPOR European Congress 2018 PMH 33-November 12, 2018 2. Kiatsangorn T, Pono K, Udombhornprabha A., Daily antipsychotics cost in schizophrenic patients at Nakorn

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AWARD RECEIVED

1. Traveling Scholarship Award for meeting, training courses and participation at the 39th Annual Meeting Congress of Society for Medical Decision Making, 21-25 October, 2017 at Pittsburgh, USA- Special Membership Fee Waiver Granted (2017-2020)
2. Research grant recipient one of the research team member for the Cluster on Aging Research of Chulalongkorn University during 2014-2016.
3. Award for Recognition: the contribution to the Pharmacological and Therapeutic Society of Thailand in 2008.
4. Award for Recognition of the academic contributor for supporting academic achievement of “Hearing International” an NGO under the campaign of WHO for better hearing for all in 2005.
5. Award for Recognition from the Psychiatric Association of Thailand & DOMH for the academic projects initiations and supports
6. Scholarship Recipient of the General Cultural Scholarship, Government of India for M.Pharm. in Pharmacology, Faculty of Medicine, Nagpur University, MS, India
7. Recipient of the exchange leader-mentor program for Chiang Mai University, Chiang Mai, Thailand & Saint Olaf College, North Field, MN, USA during 1982-1988.

8. Recipient of the –Marketing Survey Research Grants: collaboration of Department of Pharmaceutical Administration, Faculty of Pharmacy, Chiang Mai University & the Lederle