Original Article

Thai Journal of Pharmaceutical Sciences

Characterization of quality attribute and specification for standardization of acemannan extracted from *Aloe vera* for tissue regeneration

Siriwan Seeleamngam[1], Polkit Sangvanich[2], Pasutha Thunyakitpisal[3,4], Vorasit Vongsutilers[1]

[1]Department of Food and Pharmaceutical Chemistry, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand, [2]Department of Chemistry, Faculty of Sciences, Chulalongkorn University, Bangkok, Thailand, [3]Research Unit of Herbal Medicine, Biomaterial and Material for Oral Treatment, Department of Anatomy, Faculty of Dentistry, Chulalongkorn University, Bangkok, Thailand, [4]Institute of Dentistry, Suranaree University of Technology, Nakhon Ratchasima, Thailand

Corresponding Author:
Vorasit Vongsutilers,
Department of Food and Pharmaceutical Chemistry, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, 10330, Thailand.
Tel: +66-801795142.
E-mail: vorasit.v@chula.ac.th
Pasutha Thunyakitpisal,
Institute of Dentistry, Suranaree University of Technology,
Nakhon Ratchasima, 30000, Thailand.
Tel: +66-87133311.
E-mail: pasutha@sut.ac.th

Received: May 07, 2022
Accepted: July 19, 2022
Published: January 16, 2023

ABSTRACT

Acemannan extracted from *Aloe vera* was well characterized as an acetylated mannose polysaccharide which have promising clinical profile for tissue regeneration. Quality aspect of acemannan is required to develop the herbal extract for practical industrial application and reliable therapeutic use. The research aim is to investigate the qualify attributes of acemannan that have impact on its safety and efficacy. Quality of three production batches of acemannan were evaluated based on ICH quality guidelines and pharmacopeial standard. The quality aspect of acemannan were determined by analytical technique including FT-IR, SEC-HPLC, RP-HPLC, GC, and loss on drying to ensure its identify and purity. The cell proliferation assay was determined to evaluate the potency of acemannan on tissue regeneration. The physicochemical characters of acemannan samples were consistency. Their identities confirmed by IR spectra and retention time. Molecular size distribution has shown that acemannan size below 100 kD may contribute to better activity as seen in cell proliferation assay and can be used to qualify acemannan potency. The purity profile of acemannan has indicates that contamination of impurities including aloin and residual solvent were within acceptable limits. Specification of acemannan as active pharmaceutical ingredient was established and can be applied for commercial use.

Keywords: *Aloe vera*, acemannan, specification, quality control, size distribution, tissue regeneration

INTRODUCTION

Acemannan is an unique polysaccharide which can be obtained from *Aloe vera* gel extract. Prior characterization on acemannan demonstrated its mean molar mass in the range of 150–1500 kD[1,2] with mannose monomer up to 80%. The chemical structure of acemannan has been described as acetylated polymannose in the form of β-(1–4) acetylated polymannose[3,4] arranged into distinctive repeating unit which is composed of five monosaccharide molecules i.e. acetylated mannose, acetylated mannose, glucose, acetylated mannose, and mannose, respectively,[5] as shown in Figure 1. Pharmacological and potential therapeutic effects of acemannan have been demonstrated in several studies, *in vitro* and *in vivo*. Acemannan promotes soft and hard tissue healing through stimulation of collagen formation, growth factors production including vascular endothelial growth factor that accelerates angiogenesis and keratinocyte growth factor that accelerates epithelial cell proliferation and motility of epithelial cells to cover wounds.[6] Bone morphogenic proteins (BMP-2 and BMP-4) that induce bone formation in primary pulpal fibroblasts and periodontal fibroblasts can also be promoted by acemannan.[7] In addition, acemannan acts to regulate the immune response through stimulation of IL-6 and IL-8 secretion where it binds to toll-liked receptor 5 which forwards intracellular signaling pathways to NF-kappa B protein nuclei that regulates expression of IL-6 and IL-8.[8] In term of safety, a study by Fogleman *et al*.[9] determined the acute toxicity of acemannan extract in animal model including

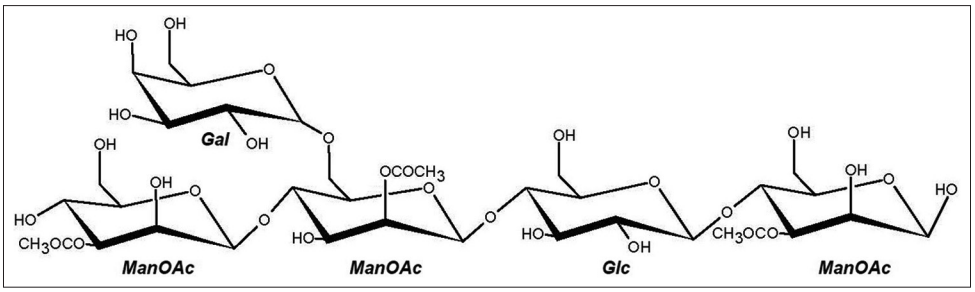

Figure 1: Chemical structure of acemannan repeating unit. Glc and Gal are glucose and galactose, while ManOAc represents acetyl mannose

rat, mice, and dog with intravenous and intraperitoneal administrations. Data obtained from both single dose toxicity and repeat dose toxicity tests demonstrated that no significant signs of toxicity and no deaths occurred in animals treated with acemannan. Clinical studies have demonstrated the effect of acemannan on acceleration of oral aphthous ulcer healing, osteodentin bridge formation, post-extraction tooth socket healing, and maxillary sinus bone augmentation[10-15] which indicates safety and efficacy of acemannan in therapeutic use. Although acemannan extracted from *Aloe vera* has been shown to have promising prospect to be applied for therapeutic use especially in wound healing due to its safety and efficacy profile, information on its quality that links to safety and efficacy are still limit. Inconsistency quality which is a common barrier for herbal extracts that restraints practicality in production of industrial scale and using them in clinical application. Therefore, in order to develop acemannan to active pharmaceutical ingredient (API) for therapeutic or health promotion usage, characterization of acemannan quality profile that reflects its safety and efficacy is mandatory. This study focused on investigation of acemannan quality attributes and development of acemannan specification that is important for its quality control such that the herbal extract can develop to meet proper standard, and appropriately progress into pharmaceutical grade which will be beneficial in bringing advancement of herbal medicine to real therapeutic use.

MATERIALS AND METHODS

Acemannan Extract

Aloe vera Linn. was purchased from a local herbal supplier in Jatujak market (Bangkok, Thailand). Three batches of acemannan extract were produced by ethanol precipitation according to previously report procedure.[16] Production scale of Batch 001, 002, and 003 were produced from 100 kg of fresh *Aloe vera* leaf gel to yield 40.1 g, 38.4 g, and 37.2 g, respectively. The off-white odorless extract powders were stored in glass vials at room temperature.

Quality Profiling of Acemannan

To profile quality attributes of acemannan, several tests have been performed to measure physical, chemical, and biological properties of the extract. Selection of the test was based on parameters that would ensure identity, content of acemannan, and potential impurities that may be presented. In addition, other relevant characteristics that can affect safety and efficacy of the extract that is planned to be used as API were included in the study.

Three batches of acemannan were studied in order to demonstrate reproducibility of each quality attribute. The analytical methods performed in this study are adapted from pharmacopeial methods or the developed and validated methods obtained in our laboratory.

Fourier-transform infrared spectroscopy (FT-IR)

FT-IR was mainly used to confirm identity of acemannan based on absorption bands corresponded to nature of acemannan that is acetylated polysaccharide. The analysis was carried out using FT-IR Spectrophotometer (Thermo scientific Nicolet iS10, United States of America [USA]). Acemannan samples were prepared by mixing acemannan 2 mg with potassium bromide 100 mg, and then potassium bromide pellets were obtained with hand press. The spectrum was recorded with wavenumber range 400–4000 cm^{-1} with 32 number of scans per spectrum

Size exclusion-high-performance liquid chromatography (SEC-HPLC)

The polysaccharide nature gives rise to the characteristic that acemannan is composed of various size of polymeric chains. Size of the active molecule may affect its pharmacological activity as observed in other therapeutic polysaccharides like heparin.[17] Therefore, determination of molecular weight distribution of acemannan is crucial for investigation of acemannan quality attribute. Molecular weight distribution analysis of acemannan extract was performed with HPLC equipped with refractive index detector (Shimadzu HPLC-RID model LC-10A, Japan). The size exclusion stationary phase is composed of multiple columns including Shodex OHpak SB-G 8 × 5 mm, Shodex OHpak SB-804 8 × 300 mm, and Shodex OHpak SB-805 8 × 300 mm to enhance separation power. The column oven was kept at 40°C. The separation was carried out under isocratic elution with 1 mM sodium azide in water as mobile phase. The flow rate was operated at 0.8 mL/min. The injection volume was 50 µL. The standard solution for molecular weight estimation was prepared using 0.2 mg/mL of each P-82 standard (P–5, P–10, P–20, P–50, P–100, P–200, P–400, and P–800, the United State Pharmacopeial, USA) in mobile phase. The retention time of each standard and its molecular weight were then used to plotted calibration curve for molecular weight estimation. Acemannan samples were prepared as 0.2 mg/mL in mobile phase. The sample is required to be stored at room temperature for 12 h for complete dissolution of sample.

Cell proliferation assay

Human gingival fibroblasts were obtained from explanted culture of gingival tissue during the surgical removal of

impacted third molar under a protocol approval by the Human Ethics Committee, Faculty of Dentistry, Chulalongkorn University. All experiments were established using cells from the third to the fifth passage. The gingival fibroblast proliferation assay was performed to demonstrate activity of acemannan on cell proliferation promotion which is one of acemannan primary effect on wound healing and tissue regeneration. The MTT assay utilizes 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide which will be reduced by mitochondrial reductase in living cells to produce product which can be detected by colorimetry.

For experiment, the cells (20,000 cell/well) were seeded in 24-well tissue culture plate and grown in growth culture media (Dulbecco's modified Eagle's media [DMEM] supplemented with 10% fetal bovine serum; Gibo, Life Technologies, NY, USA), and incubated at 37°C and 5% CO_2 for 24 h. The cells were treated with acemannan extract at concentration range from 0, 0.5, 1.0, 2.0, and 4.0 mg/mL in serum free DMEM media for another 24 h. After incubation, the cells were washed with phosphate buffered saline (PBS) and incubated with 1 mg/mL MTT solution for 30 min. The cells were washed with PBS and DMSO was then added to stop the reaction. The absorbance was then determined at a wavelength of 570 nm using microplate reader (EPOCHTM; Agilent, USA). The numbers of cell viability were calculated using a standard curve. The experiment was 3-times independently repeated.

Loss on drying

Acemannan contains considerable amount of water and other solvent due to nature of its origin and manufacturing process that involves the use of water and solvent. Therefore, the amount of moisture in acemannan is an important piece of information regarding the control of its hydrolysis degradation and microbial contamination. Among several water determination methods, loss on drying has been used to determine water content in acemannan. The procedure starts with weigh about 100 mg of acemannan extract in an evaporating dish, then dry at 105°C for 3 h in hot air oven (Binder hot air oven model ED260, Germany). Continue the drying and weighing every hour intervals until constant weight. Calculate percentage of the weight loss compared to the original weight of acemannan sample.

Residual solvent determination

Due to our manufacturing process of acemannan that involves ethanol extraction, there is the risk of leftover residual solvent remain in the extract. Determination of potential solvent contamination, therefore, has been performed. Ethanol is the main risk of contamination, while methanol and benzene are common impurities in ethanol so they were included in analysis. Methanol, ethanol, and benzene standards were obtained from Chem service Inc. (West Chester, PA, USA). Analytical method for residual solvent determination in acemannan extract has performed by gas chromatography with flame-ionization detector, using Shimadzu Nexis GC-2030 Injector coupling with Shimadzu Headspace HS-20. The running condition includes oven temperature at 80°C, equilibration time at 30 min, sample line temperature 110.0°C, transfer line temperature 120.0°C, pressurization time for 1 min, and injection volume is 1 mL with split ratio of 1:5. Helium was used as the carrier gas at a linear velocity of 35.0 cm per second. The stationary phase is Agilent J&W GC Column DB-624 30-m × 0.530-mm × 3.00-μm. The chromatographic condition was programmed to maintain the column temperature at 40°C for 20 min, then increase the temperature at a rate of 10°C per min to reach 240°C and maintain at this temperature for 20 min. The injection port temperature was maintained at 140°C and the flame-ionization detector temperature was maintained at 260°C. The sample preparation was prepared by transfer 50 mg of acemannan extract into GC vial, and then add 5 mL of water to GC vial. The standard solution was prepared to contain 20 ppm of methanol, 20 ppm of ethanol, and 2 ppm of benzene. Amount of residual solvent in acemannan sample was quantify from sample signal compared with standard signal of each solvent.

Aloin determination

Aloin is anthraquinone found in *Aloe vera*. It is a main composition in Aloe[18] which contributes to laxative effect.[19] Although aloin's physicochemical properties are quite different from acemannan and it should be eliminated during acemannan extract and purification, early development of acemannan production has been show that small amount of aloin can contaminate acemannan extract which contributes to coloration of the extract and may contribute to unwanted laxative effect for acemannan use in wound healing. Aloin standard used in this study was obtained from Sigma-Aldrich (St. Louis, MO, USA). Analysis of aloin in acemannan was performed by reverse-phase HPLC. Shimadzu HPLC LC-10A with stationary phase Inertsustain 4.6-mm × 150-mm, 5 μm column was used to performed analysis. The column oven was operated temperature at 43°C. The separation was carried out under isocratic elution with 30% acetonitrile in water as mobile phase with flow rate maintained at 1.0 mL/min. The injection volume was 20 μL and the detection were analyzed using UV at 295 nm. The standard preparation contained 1 μg/mL of aloin in 50% methanol. The sample solution contained acemannan 0.5 mg/mL was prepared as follows. Transfer 12.5 mg of acemannan extract into 25-mL volumetric flask, add 8 mL of methanol and swirl until completely dispersed. Add 8 mL of water then sonicate for 30 min and allow to cool at room temperature for 5 min. Dilute to volumetric with 50% methanol. Centrifuge the acemannan sample solution at 17,000 rpm for 30 min at 4°C. Filter the supernatant through 0.45-micron nylon membrane and analyze the filtrate. Amount of aloin in acemannan was determined from aloin signal in sample compared with standard signal.

Justification of Acemannan Specification

Base on quality analyses that provide quality attributes of acemannan, compilation of the data including various tests was used to justify acemannan specification. The International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) quality guidelines were applied to set specification of acemannan for the purpose of using it as drug substance. Mainly, ICH Q6A Specifications: Test Procedures and Acceptance Criteria for New Drug Substances and New Drug Products: Chemical Substances[20] was implemented for comprehensive scope of specification necessary for acemannan quality control, while ICH Q3A

Impurities in New Drug Substances,[21] and ICH Q3C Guideline for Residual Solvents[22] were used to justify risk and set limit of relevant impurities in acemannan.

RESULTS AND DISCUSSION

To develop herbal extract to be used as API or drug substance requires considerate amount of evidence to ensure that certain substance is efficacious and has tolerable toxicity. One of the biggest barriers that hindrances translation of the substance originated from medicinal herbal to be practical for therapeutic use is the lack of suitable quality control that connected to its safety and efficacy. Acemannan actually has been widely studied for its pharmacological effects and structure characterization but there is limit information on quality attributes that necessary to ensure reproducibility of the claimed activities. In this study, quality analyses have been performed in accordance with international standards for pharmaceutical use and specification for quality control of acemannan has been justified and proposed for exploiting the extract to reach pharmaceutical grade that can further develop for therapeutic use.

Physical appearance is a very simple test to perform to discriminate obvious contamination or adulteration before going through more extensive laboratory analysis. Based on observation, acemannan extract is a solid powder with white to off-white color in nature. Discoloration such as yellowish powder indicates contamination of impurities in unacceptable level. Therefore, appearance of extract was justified as one of the specifications that need to be assessed first. Acemannan extract is odorless and tasteless, however for safety purpose; these characteristics are omitted from specification and routine quality control testing. Identity is a very important attribute that directly connect to pharmacological effect of acemannan. FT-IR provides structural information in form of specific absorption bands in IR spectrum. Acemannan structure contains acetyl group that show absorption bands at 1740 and 1250 cm^{-1} which associated with C = O and C-O-C stretching, respectively. The abundance of -OH groups from sugar residues give strong broad band of OH stretching around 3400 cm^{-1}. Sugar ring skeleton that contains O-C-O also give rise to medium bands at 1650 and 1380 cm^{-1}. IR spectra obtained from three batches of acemannan extracts, as shown in Figure 2, match with previously well characterized acemannan[5] and show characteristic bands at 1740 and 1250 cm^{-1} which will be used as acceptance criteria for FT-IR identification. Since there is no reference standard grade of acemannan available, acceptance criteria based on characteristic bands is preferable and more practical for routine work at this point compared to overall spectrum comparison which requires reference standard or reference spectrum. Development of acemannan reference standard is ongoing in our laboratory and will be utilized for the extract quality control in the future which would improve specificity of the identification test. Sugar composition such as mannose and acetylate mannose was characterized previously in our structure elucidation phase[5] and can serve as information for identification. Nevertheless, nature of the test that requires tedious sample digestion renders its suitability for routine test. Although there are other analysis methods used for full structure characterization such as ^{1}H-NMR, ^{13}C-NMR, or MS, the nature of identification test is confirmatory which FT-IR in combination with SEC-HPLC serve the purpose and it is the analytical instrument that more practical for routine quality control test. In addition, in conjunction with cell-based activity assay which demonstrates unique pharmacological activity of acemannan, the identity of acemannan would be sufficiently confirmed. This concept of identification is seen in

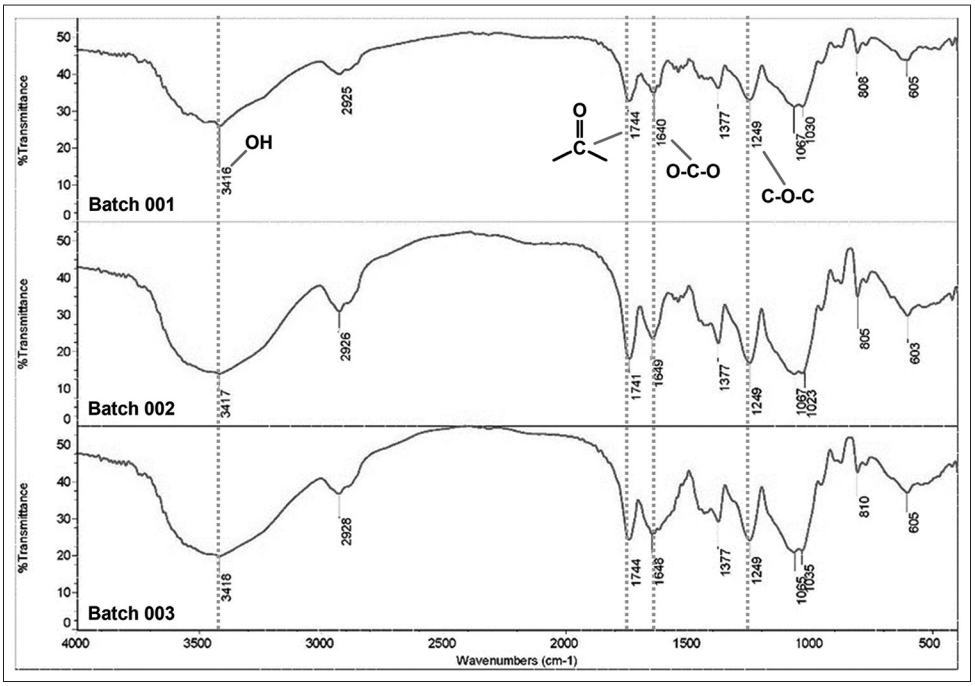

Figure 2: Fourier-transform infrared spectroscopy for acemannan identification. IR spectra from three batches of acemannan shown similar characteristic bands around 3400, 1740, 1650, and 1250 cm-1

pharmacopeial monograph of polysaccharide drug like heparin sodium[23] that does not identify subunit nor sugar residue but utilizing combination of spectroscopy, chromatography, and biological assay for identification.

Several studies has proposed molecular weight of acemannan in the range of couple hundreds to thousands kD.[1] Nevertheless, none has report on acemannan molecular size distribution and this characteristic may contribute to pharmacological effect of acemannan. SEC-HPLC has been performed to investigate distribution in size of acemannan. The result indicates that majority of acemannan are in molecular weight range above 1000 kD (A1), while the rest have molecular weight below 100 kD (A2) [Figure 3].

Three batches of acemannan provide similar trend with remark that batch 003 has molecular weight distributed to smaller size acemannan more than other two batches. The peak area ratio between acemannan molecular weight below 100 kD and acemannan molecular weight above 1000 kD (A2/A1 ratio) are 0.10, 0.14, and 0.41 for batches 001, 002, and 003, respectively. In conjunction with cell proliferation assay, acemannan batch 003 provide the highest activity for stimulation of gingival fibroblast proliferation as shown in Figure 4. The 10% serum supplement culture media has been recommended as a growth culture media for mammalian cells. Serum contains a mixture of nutrients, proteins, hormones, and growth factors that is important for cell activity.[24] Therefore, the cells which cultures in serum free media and 10% serum supplement media were considered as control and positive control, respectively, to compare the mitogenic activity of acemannan.

This observation provides important piece of information that molecular weight distribution plays role in activity of acemannan and specification can be derived from this result. Although there is room for further study on relationship between acemannan size and activity, the current criteria for quality control of acemannan is that the A2/A1 ratio is not <0.10 based on the result from batch 001 that have lowest activity. Together with cell proliferation assay, SEC-HPLC analysis serves as a quality control test to ensure wound healing effect of the extract. In addition, identification with retention times of major components from chromatographic profile is also complemented with FT-IR identification.

Water content in acemannan extract was determined by loss on drying. Karl Fischer coulometric titration that usually uses for water determination in drug substances is not a practical choice due to acemannan swelling nature that may prevent solvent extraction of water from its mass; hence, this method may give inaccurate measurement. The result from loss on drying test shows consistency in all three batches which have % loss on drying around 11% as shown in Table 1. The acting specification for the extract therefore is set at 12%. Further stability study will provide additional information regarding the suitable level of %loss on drying which may gain or loss during storage in relation to other quality attributes that are impacted by %loss on drying such as microbial contamination. Acemannan has been demonstrated to have high safety profile in animal study[9] However, impurities contamination may also contribute to unwanted toxicity. Residual solvent including ethanol, methanol, and benzene are included in analysis which

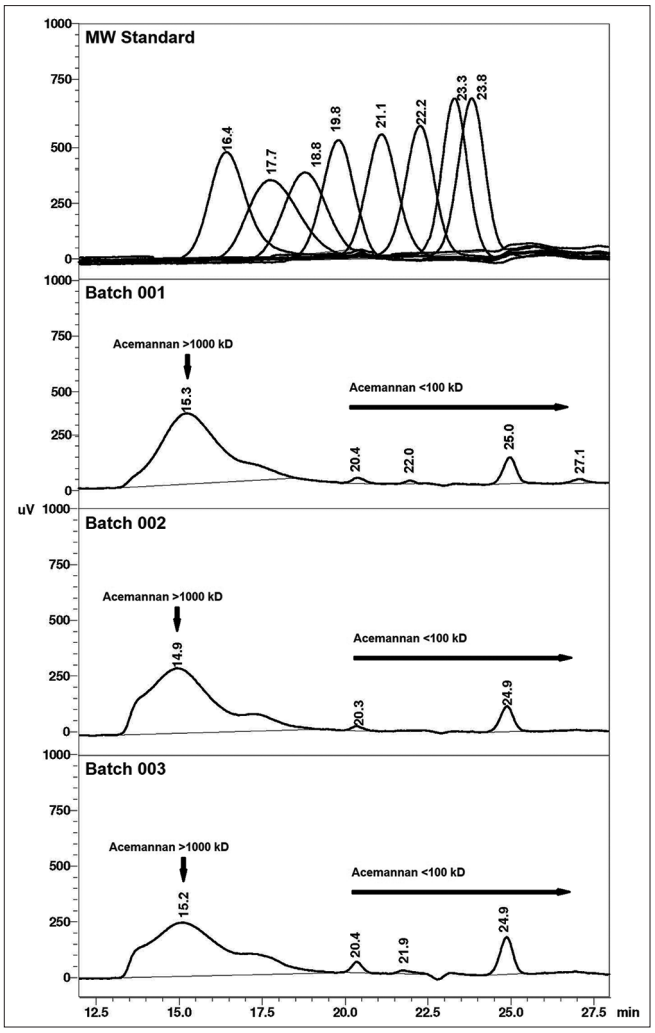

Figure 3: Size exclusion-high-performance liquid chromatography chromatogram of acemannan. Three batches of acemannan are mainly composed of acemannan molecular weight above 1000 kD as shown in chromatogram at retention time around 15 min., while batch 003 has higher amount of acemannan molecular weight below 100 kD which contains peaks at retention time more than 20 min

results as shown in Table 1. Both ethanol and methanol are in acceptable limit based on permitted daily exposure published in ICH Q3C guideline, while benzene, a carcinogenic class 1 solvent,[22] was not detected in all batches of acemannan indicates that acemannan extracted from current process has low risk of residual solvent contamination. Aloin was also not detected in all batches of acemannan and its acceptance criteria was determined according to ICH Q3A qualification threshold considering the worst case scenario that maximum daily dose of acemannan is above 2 g. In addition to quality tests performed in this study, there are risk that acemannan extract may be contaminated with microbial contamination due to its raw material origin that came from soils. Although the manufacturing process uses ethanol extraction, microbial contamination can also occur during downstream process and storage. The polysaccharide nature of acemannan and moisture of the extract are likely sources for growth of microbial organisms. Therefore, there is significant risk of microbial contamination that should be controlled. Acceptance criteria

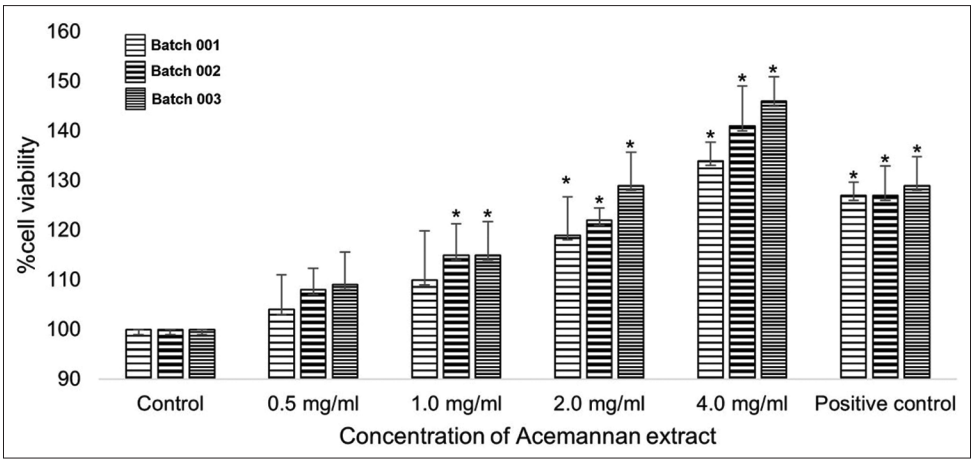

Figure 4: The result of gingival fibroblast proliferation assay obtained with three batches of acemannan. *Indicates the significant difference compared with control group ($P<0.05$). Control: Serum free Dulbecco's modified Eagle's media (DMEM) culture media; Positive control: 10% serum supplemented DMEM culture media

Table 1: Analysis results from loss on drying, residual solvents, and aloin content determination

Acemannan	Analytical testing				
	Loss on drying (%)	Residual solvents			Aloin content (%)
		Ethanol (ppm)	Methanol (ppm)	Benzene (ppm)	
Batch 001	10.7 (8.0)	138 (2.4)	501 (2.7)	ND	ND
Batch 002	10.5 (1.4)	88 (0.3)	496 (0.1)	ND	ND
Batch 003	10.6 (5.9)	137 (1.3)	492 (1.7)	ND	ND

ND: Not detect; the number in parenthesis is %RPD; ICH Q3C guideline permits the acceptable limit of ethanol, methanol, and benzene contaminations at level of 5000, 3000, and 2 ppm, respectively

Table 2: Acemannan extract specification and justification of specification

Tests	Acceptance criteria	Justification
1. Appearance	White to off-white fine powder	Physical appearance observation that helps identity and purity screening
2. Identification	IR spectrum shows characteristic absorption bands at wavenumbers around 3400, 1740, 1650, and 1250 cm^{-1} Chromatogram shows major peak at retention time at about 15 and 25 min The activity of acemannan is NLT the positive control	Three complementary identification methods to ensure identity of acemannan based on its specific IR absorption, chromatographic profile, and biological activity
2.1 FT-IR		
2.2 SEC-HPLC		
2.3 Cell proliferation activity		
3. Molecular size distribution	The peak area ratio of acemannan molecular weight below 100 kD and acemannan molecular weight above 1000 kD is NLT 0.1	Base on analysis of the investigated batches. The distribution of molecular size reflects acemannan on cell proliferation promotion.
4. Cell proliferation assay	The activity of acemannan is NLT the positive control	Bioassay to ensure therapeutic effect of acemannan
5. Loss on drying	NMT 12%	Base on analysis of the investigated batches
6. Residual solvent		Base on permitted daily exposure of individual solvent described in ICH Q3C
6.1 Ethanol	5000 ppm	
6.2 Methanol	3000 ppm	
6.3 Benzene	2 ppm	
7. Aloin content	0.05%	Base on qualification threshold of organic impurities described in ICH Q3A
8. Microbial contamination		Base on potential therapeutic use of acemannan for wound healing in oromucosal and gingival route. The acceptance criteria are met with USP general chapter<1111>
8.1 TAMC	10^2 cfu/g	
8.2 TYMC	10^1 cfu/g	
8.3 *Escherichia coli*	Absence/g	
8.4 *Staphylococcus aureus*	Absence/g	
8.5 *Pseudomonas aeruginosa*	Absence/g	

NLT: Not less than, NMT: Not more than, CFU: Colony forming unit, FT-IR: Fourier-transform infrared spectroscopy, SEC-HPLC: Size exclusion-high-performance liquid chromatography, TYMC: Total combined yeasts and molds count, TAMC: Total aerobic microbial count

for microbial contamination has been set to be compiled with USP general chapter <1111>.[25] The therapeutic purpose of acemannan extract in this study is wound healing especially for oral and gingival mucosa that have previous clinical evidence support the use, thus the acceptance criteria for microbial quality are following. The total aerobic microbial count is not more than 10^2 cfu/g and the total combined yeasts and molds count is not more than 10^1 cfu/g, while specified pathogenic microorganism including *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* must be absent from acemannan extract.

A complete list of specification of acemannan for pharmaceutical use in oral wound healing and tissue regeneration is proposed in Table 2 along with brief justification for specification. The designated tests demonstrate quality attributes that reflect efficacy and safety of acemannan extract that can be used for quality control in routine lab work. Stability study of acemannan extract is the next step to determine the shelf-life of the extract as well as to perfect the specification of acemannan to be used as potential drug substance.

CONCLUSION

Quality attribute characterization of acemannan extracted from *Aloe vera* by physicochemical and biological tests including identification, molecular size distribution, loss on drying, determination of impurities, and cell proliferation assay has shown that the extract met with acceptable quality to reflect safety and efficacy for its use as pharmaceutical substance for tissue regeneration. Three batches of acemannan were studied and demonstrated reproducibility of each quality attribute. Further study including industrial scale manufacturing and stability study will be necessary to achieve pharmaceutical grade of acemannan extract that is commercial ready. Specification established in this study can be used as an acting standard for quality control purpose of acemannan manufactured as a drug substance.

ACKNOWLEDGMENTS

This research is funded by Chulalongkorn University grant number CU-GR-63-01-33-01 and TSRI Fund (CU_FRB640001_32_2).

REFERENCES

1. Liu C, Cui Y, Pi F, Cheng Y, Guo Y, Qian H. Extraction, purification, structural characteristics, biological activities and pharmacological applications of acemannan, a polysaccharide from *Aloe vera*: A Review. Molecules 2019;24:1554.
2. Miramon-Ortíz DA, Argüelles-Monal W, Carvajal-Millan E, López-Franco YL, Goycoolea FM, Lizardi-Mendoza J. Acemannan gels and aerogels. Polymers (Basel) 2019;11:330.
3. Ni Y, Turner D, Yates KM, Tizard I. Isolation and characterization of structural components of *Aloe vera* L. leaf pulp. Int Immunopharmacol 2004;4:1745-55.
4. Talmadge J, Chavez J, Jacobs L, Munger C, Chinnah T, Chow JT, *et al*. Fractionation of *Aloe vera* L. inner gel, purification and molecular profiling of activity Int Immunopharmacol 2004;4:1757-73.
5. Chokboribal J, Tachaboonyakiat W, Sangvanich P, Ruangpornvisuti V, Jettanacheawchankit S, Thunyakitpisal P. Deacetylation affects the physical properties and bioactivity of acemannan, an extracted polysaccharide from *Aloe vera*. Carbohydr Polym 2015;133:556-66.
6. Jettanacheawchankit S, Sasithanasate S, Sangvanich P, Banlunara W, Thunyakitpisal P. Acemannan stimulates gingival fibroblast proliferation; Expressions of keratinocyte growth factor-1, vascular endothelial growth factor, and Type I collagen; and wound healing. J Pharmacol Sci 2009;109:525-31.
7. Jittapiromsak N, Jettanacheawchankit S, Lardungdee P, Sangvanich P, Thunyakitpisal P. Effect of acemannan on BMP-2 expression in primary pulpal fibroblasts and periodontal fibroblasts, *in vitro* study. J Oral Tissue Eng 2007;4:149-54.
8. Thunyakitpisal P, Ruangpornvisuti V, Kengkwasing P, Chokboribal J, Sangvanich P. Acemannan increases NF-κB/DNA binding and IL-6/-8 expression by selectively binding Toll-like receptor-5 in human gingival fibroblasts. Carbohydr Polym 2017;161:149-57.
9. Fogleman RW, Chapdelaine JM, Carpenter RH, McAnalley BH. Toxicologic evaluation of injectable acemannan in the mouse, rat and dog. Vet Hum Toxicol 1992;34:201-5.
10. Bhalang K, Thunyakitpisal P, Rungsirisatean N. Acemannan, a polysaccharide extracted from *Aloe vera*, is effective in the treatment of oral aphthous ulceration. J Altern Complement Med 2013;19:429-34.
11. Deesricharoenkiat N, Jansisyanont P, Chuenchompoonut V, Mattheos N, Thunyakitpisal P. The effect of acemannan in implant placement with simultaneous guided bone regeneration in the aesthetic zone: A randomized controlled trial. Int J Oral Maxillofac Surg 2022;51:535-44.
12. Vu NB, Chuenchompoonut V, Jansisyanont P, Sangvanich P, Pham TH, Thunyakitpisal P. Acemannan-induced tooth socket healing: A 12-month randomized controlled trial. J Dent Sci 2021;16:643-53.
13. Vu TT, Nguyen MT, Sangvanich P, Nguyen QN, Thunyakitpisal P. Acemannan used as an implantable biomaterial for vital pulp therapy of immature permanent teeth induced continued root formation. Pharmaceutics 2020;12:644.
14. Le Van C, Thu HP, Sangvanich P, Chuenchompoonut V, Thunyakitpisal P. Acemannan induces rapid early osseous defect healing after apical surgery: A 12-month follow-up of a randomized controlled trial. J Dent Sci 2020;15:302-9.
15. Trinh HA, Dam VV, Le B, Pittayapat P, Thunyakitpisal P. Indirect sinus augmentation with and without the addition of a biomaterial: A randomized controlled clinical trial. Implant Dent 2019;28:571-7.
16. Jittapiromsak N, Sahawat D, Banlunara W, Sangvanich P, Thunyakitpisal P. Acemannan, an extracted product from *Aloe vera*, stimulates dental pulp cell proliferation, differentiation, mineralization, and dentin formation. Tissue Eng Part A 2010;16:1997-2006.
17. Lemke TL, Williams DA, Roche VF, Zito SW. Foye's Principles of Medicinal Chemistry. 7th ed. Netherlands: Wolters Kluwer Health Adis (ESP); 2012.
18. The United State Pharmacopeia. Aloe. United States: The United State Pharmacopeia; 2022.
19. Bokelmann JM. 24-Aloe (*Aloe vera, Aloe barbardensis*): Leaf and leaf pulp. In: Bokelmann JM, editor. Medicinal Herbs in Primary Care. Netherlands: Elsevier; 2022. p. 179-87.
20. The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH). In: International Conference on Harmonization: Q6A-Specifications : Test Procedures and Acceptance Criteria for New Drug Substances and New Drug Products: Chemical Substances. Europe: Europe the Middle East and Africa; 1999.
21. The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH). ICH Q3A(R2)-Impurities in New Drug Substances; 2006.
22. The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH). ICH

Q3C(R8)-Guideline for Residual Solvents; 2021.
23. The United State Pharmacopeia. Heparin Sodium. United States: The United State Pharmacopeia; 2022.
24. Yang Z, Xiong HR. Culture conditions and types of growth media for Mammalian cells. In: Ceccherini-Nelli L, Matteoli B, editors. Biomed Tissue Cult. Rijeka: IntechOpen; 2012.
25. The United State Pharmacopeia. <1111> Microbiological Examination of Nonsterile Products: Acceptance Criteria for Pharmaceutical Preparations and Substances for Pharmaceutical Use. United States: The United State Pharmacopeia; 2022.