STUDY OF THAI WOOD VINEGAR AS ANTIBIOTIC ALTERNATIVE ON DIARRHEA PREVENTION AND GROWTH PERFORMANCE IN WEANED PIGLETS

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การศึกษาใช้น้ำส้มควันไม้ไทยเพื่อเป็นยาปฏิชีวนะทางเลือกในการป้องกันท้องเสียและประสิทธิภาพการเจริญเติบโตในลูกสุกรหย่านม

นางสาวพิมพ์ชนก โล่ห์ทองคำ

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต
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Suranaree University of Technology has approved this thesis submitted in partial fulfillment of the requirements for the Degree of Doctor of Philosophy.

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น้ำส้มควันไม้เป็นผลพลอยได้จากการผลิตถ่านไม้ สารประกอบต่างๆ ในไม้ฟืนจะถูกสลายตัวด้วยความร้อนเกิดเป็นสารประกอบอินทรีย์ใหม่ที่เชื่อว่ามีความสามารถในการยับยั้งแบคทีเรีย ทั้งนี้คุณสมบัติจะแตกต่างกันไปขึ้นกับชนิดของไม้ฟืนและกระบวนการผลิต ดังนั้นวัตถุประสงค์ของการศึกษานี้คือ ศึกษาคุณสมบัติการเป็นยาปฏิชีวนะของน้ำส้มควันไม้มะขาม (*Tamarindus indica*), น้ำส้มควันไม้มะม่วง (*Mangifera indica*) และน้ำส้มควันไม้สะเดา (*Azadirachta indica*) โดยศึกษา 1) องค์ประกอบทางเคมี 2) ฤทธิ์ในการยับยั้งเชื้อแบคทีเรีย 3) ผลต่อประสิทธิภาพการเจริญเติบโต, จำนวนประชากรแบคทีเรียในมูลลูกสุกร, รูปร่างของวิลไลในลำไส้เล็ก และค่าโลหิตวิทยาในลูกสุกรหย่านม ผลการศึกษาพบว่า น้ำส้มควันไม้มีค่าความเป็นกรด (pH) อยู่ระหว่าง 3-4 การศึกษาองค์ประกอบทางเคมีโดยใช้เครื่อง GC-MS แสดงถึงสารประกอบหลักในน้ำส้มควันไม้คือ กรดอะซิติก มีปริมาณ 18.6-29.45% และสารประกอบอื่นๆได้แก่ ฟีนอล, อัลดีไฮด์, แอลกอฮอล์ รวมถึงอนุพันธ์อื่นๆ ซึ่งส่วนประกอบหลายชนิดเหล่านี้ส่งผลยับยั้งเชื้อแบคทีเรียโดยออกฤทธิ์วงกว้าง (*Staphylococcus aureus* TISTR 746, *Escherichia coli* TISTR 073, *S. aureus* ATCC29213, Methicillin-resistant *S. aureus* and *E. coli* ATCC25922) โดยค่าความเข้มข้นในระดับต่ำสุดที่สามารถยับยั้งการเจริญของเชื้อแบคทีเรีย (MIC) เท่ากับ 1.56-6.25 %v/v และทำให้เซลล์แบคทีเรียสูญเสียองค์ประกอบภายในเซลล์ส่งผลต่อรูปร่างที่ผิดรูปกว่ากลุ่มควบคุม นอกจากนี้ผลการเสริมน้ำส้มควันไม้มะขาม และน้ำส้มควันไม้มะม่วงในลูกสุกรหย่านม สามารถลดจำนวนประชากรแบคทีเรียรวมในมูลลูกสุกรในวันที่ 28 ได้ดีกว่ากลุ่มควบคุม (P<0.05) ถึงแม้ว่าน้ำส้มควันไม้ทั้ง 3 ชนิดจะมีผลต่อประสิทธิภาพการเจริญเติบโตในลูกสุกรหย่านมไม่แตกต่างจากกลุ่มควบคุม แต่น้ำส้มควันไม้มะขาม และน้ำส้มควันไม้มะม่วง สามารถเพิ่มความสูงของวิลไลในลำไส้เล็ก และส่งผลต่อสัดส่วนระหว่างความสูงของวิลไลกับความลึกของคริปท์เซลล์ในลำไส้เล็กส่วนดูโอดีนัม, เจจูนัมและไอเลียมมีค่าสูงกว่ากลุ่มควบคุม (P<0.05) ซึ่งสอดคล้องกับภาพถ่ายจากกล้องจุลทรรศน์อิเลคตรอน บริเวณส่วนปลายของวิลไลในลำไส้เล็กส่วนดูโอดีนัมและเจจูนัม ที่พบเซลล์เยื่อบุผิวไมโครวิลไลที่มีขนาดใหญ่ส่งผลให้ไมโครวิลไลยาวขึ้น อีกทั้งการเสริมน้ำส้มควันไม้ในลูกสุกรไม่ส่งผลต่อค่าโลหิตวิทยาและค่าเคมีในเลือด ดังนั้นจากข้อมูลทั้งหมดสามารถสรุปได้ว่าน้ำส้มควันไม้มะขาม และน้ำส้มควันไม้มะม่วงมีคุณค่าเชิงการแพทย์ที่ออกฤทธิ์ยับยั้งแบคทีเรีย และเพิ่ม

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สาขาวิชาเทคโนโลยีและนวัตกรรมทางสัตว์ ลายมือชื่อนักศึกษา Pimchanok Lohtongkam
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PIMCHANOK LOHTONGKAM : STUDY OF THAI WOOD VINEGAR AS ANTIBIOTIC ALTERNATIVE ON DIARRHEA PREVENTION AND GROWTH PERFORMANCE IN WEANED PIGLETS. THESIS ADVISOR : ASST. PROF. PAKANIT KUPITTAYANANT, Ph.D. (DVM), 126 PP.

WOOD VINEGAR/WEANED PIGLETS/ANTIBACTERIAL ACTIVITY/GROWTH PERFORMANCE/INTESTINAL MORPHOLOGY/FECAL BACTERIA

Wood vinegar is a by-product from charcoal production. It is a complex mixture of organic compounds which exhibits antimicrobial activities. Wood vinegar compositions and yields depend on process conditions and compositions of starting materials. Therefore, the purposes of this study were to explore the antibiotic properties of *Tamarindus indica*, *Mangifera indica* and *Azadirachta indica* wood vinegars by observing 1) chemical composition; 2) the antibacterial activities; 3) the effect of supplementing wood vinegars on growth performance, fecal bacterial population, intestinal villus morphology and hematology in weaned piglets. The results showed pH values of wood vinegars were 3 to 4. The GC-MS analysis showed the presence of key bioactive compounds of wood vinegars, especially acetic acid with a range of 18.6-29.45%. The others compositions were phenols, aldehydes, alcohols and others derivatives. These compounds exhibited a wide range of antibacterial activity (*Staphylococcus aureus* TISTR 746, *Escherichia coli* TISTR 073, *S. aureus* ATCC29213**,** Methicillin-resistant *S. aureus* and *E. coli* ATCC25922) indicated by the wood vinegars exhibiting broad spectrum inhibition. The MIC values were ranging from 1.56 to 6.25 %v/v. Microstructural observations showed the

depletion of the content of bacterial cells in the wood vinegars, the treated cells showed an incomplete and deformed shape when compared with the untreated cells. The study of the wood vinegars on the fecal bacterial population, the piglets treated with *T. indica* and *M. indica* wood vinegars had reduced the fecal total bacteria concentration on day 28 ($P< 0.05$). There were no effects of wood vinegar and acid supplementation on growth performance but *T. indica* and *M. indica* improved intestinal morphology by enhancing villus height and VH:CD ratio in duodenum, jejunum and ileum ($P<0.05$). In the SEM observations, very large protuberated epithelial cells and microvilli were found on the duodenal and jejunal tip surface. The hematological traits and chemical profile of piglets fed the experimental treatments were normal ranges. These findings illustrated that *T. indica* and *M. indica* wood vinegars will be beneficial medicinal plants that encourages favorable antibacterial activities and possibly be used to increase the efficiency of intestinal absorption of weaned piglets. The possible mechanisms are mainly due to its acidic action of presented phytochemical constituents including acetic acid and phenols others derivatives.

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Academic Year 2019 Advisor's Signature P. Kupittayanant

Co-advisor's Signature

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CONTENTS

Page

CONTENTS (Continued)

Page

CONTENTS (Continued)

Page

CONTENTS (Continued)

Page

CONTENTS (Continued)

Page

CONTENTS (Continued)

Page

LIST OF TABLES

Table **Page**

LIST OF TABLES (Continued)

Table **Page**

LIST OF FIGURES

Figure **Page**

LIST OF FIGURES (Continued)

Figure **Page**

LIST OF ABBREVIATIONS

ALT	=	alanine transaminase
ANOVA	=	analysis of variance
AMPs	=	antimicrobial peptides
AST	=	aspartate transaminase
ATCC	=	american type culture collection
BUN	=	blood urea nitrogen
CFU	=	colony forming unit
CP	=	crude protein
CRF	=	corticotropin-releasing factor
EDTA	=	ethylenediamine tetra-acetic acid
EHEC	=	enterohemorrhagic *escherichia coli*
ETEC	=	enterotoxigenic *escherichia coli*
ENS	=	enteric nervous system
EU	=	european union
GALT	=	gut-associated lymphoid tissue
GC-MS	=	gas chromatography mass spectroscopy
GIT	=	gastrointestinal tract
IECs	=	intestinal epithelial cells
IgA	=	immunoglobulin a
IL	=	interleukin

LIST OF ABBREVIATIONS (Continued)

LM	=	light microscope
$MgSO_4$	=	magnesium sulfate
MH	=	mueller–hinton medium
MBCs	=	minimal bactericidal concentrations
MCH	=	mean corpuscular hemoglobin
MCHC	=	mean corpuscular hemoglobin concentration
MCV	=	mean corpuscular volume
MICs	=	minimal inhibitory concentrations
MRSA	=	meticillin-resistance strain of s*taphylococcus aureus*
MSSA	=	meticillin-susceptible strain of *staphylococcus aureus*
N	=	nitrogen
NaCl	=	sodium chloride
NIST	=	the national institute of standards and technology
NRC	=	the national research council
NVFA	=	non-volatile fatty acids
PWD	=	post-weaning diarrhea
HCl	=	hydrochloric acid
HPA	=	hypothalamic pituitary adrenal axis
rRNA	=	ribosomal RNA
SCFA	=	short chain fatty acid
SEM	=	scanning electron microscope
SPSS	=	statistical package for the social science for windows

LIST OF ABBREVIATIONS (Continued)

SFB	=	segmented filamentous bacteria
sIgA	=	secretory immunoglobulin a
RBC	=	red blood cells
RCBD	=	randomized complete block design
TH	=	T helper cell
TISTR	=	the Thailand institute of scientific and technological research
TLR	=	toll like receptor
UK	=	the united kingdom
USA	=	the united states of America
VFA	=	volatile fatty acid
vv	=	volume/volume
WBC	=	white blood cells
ZO	=	zonula occludens proteins

CHAPTER I

INTRODUCTION

1.1 Rationale of the study

Antibiotics have been used as therapeutic and growth promoting agents and this has lead to improvements in the performance of animals. The practice of feeding sub-therapeutic doses was very successfully adopted and became an integral part of developing nutritional strategies for all farm livestock. The primary effects associated with the inclusion of anti-microbial feed additives are prevention of digestive disturbances, improved feed utilization and improved animal performance. Secondary effects include reduced nutrient waste, diminished environmental impact and reduced production costs. The increased use of antibiotics has given rise to a fear of the development of resistant pathogenic bacterial strains and residual contamination of the food chain with antibiotics. This has led to the adoption of safety measures and a gradual withdrawal of antibiotic promoters from pig diets. In 2006, the use of antibiotics as growth promoters was forbidden in the EU (Chen et al., 2005).

Regarding the fact that weaning time greatly affects general health condition of piglets, it is necessary to stimulate the indigenous intestinal microflora and keep it well-balanced because it provides protection to animals against invasion by pathogenic microorganisms (Jensen, 1998). These are added antibiotic to feed for piglets from birth to weaning with the aim of improving the composition of intestinal microflora in piglets and thus ameliorate the potential consequences of Post-weaning

diarrhea (Sorensen et al., 2009). It is generally accepted that the species of microorganisms in the intestinal tract and their ratio have a major impact on animal health. Enterotoxigenic *Escherichia coli* (ETEC) is the most common etiologic agent of enteric disease in piglets, accounting for the high mortality that occurs annually. Exposure to pathogens results in release of proinflammatory cytokines which activate the immune system. The activation of the immune system produces an alteration in the metabolic processes (Spurlock, 1997), resulting in suppression of protein synthesis and stimulation of muscle protein degradation (Zamir et al., 1994). Pigs experiencing such immune activation will exhibit poor performance including reduced feed intake. In-feed antibiotics have been used as growth promotants and for therapeutic treatment of gastrointestinal disease in newly weaned piglets.

However, There has been increasing concern that the use of antibiotics in food producing animals, particularly their long term use for growth promotion, contributes to the emergence of antibiotic resistant bacteria in animals. This will have an effect on performance and will almost certainly necessitate the development of new feeding, management and healthcare strategies. Plant extracts and plant compounds are new interest as antibacterial agents. The scientists have a lot of work in developing natural products extracted from plants, to produce more cost effective remedies that are affordable to the population. The acceptance of traditional medicine as alternative form of health care and the development of microbial resistance to the available antibiotics has provided to search for new antimicrobial substances from various sources like medicinal plants (Buwa et al., 2006).

Wood vinegar is a complex mixture of 80-90% water, and 10-20% organic compounds. It contains several phenolic compounds such as guaiacol and cresol, and

organic acids like acetic, formic and propionic acids. The addition of organic acids like citric, fumaric, formic and propionic acid to the diets of pigs is one of the most widely used alternative for antibiotics and has been reported to improve their performance. Their effects have been related to reduction in the growth of coliform bacteria, known to be involved in digestive disorders (Partanen and Mroz, 1999). Wood vinegar was a source of acids help to promote acidity in intestine, resulting in inhibition the growth of pathogenic bacteria, increase nutrient absorption, and reduce diarrhea (Pangnakorn et al., 2011). Wood vinegar compositions and yields depend on process conditions and compositions of starting material. Its might present different levels of bioactivity and different quantities of bioactive constituents. Many different sources of wood vinegar have been recognized as safe, natural inhibitors with various bioactivities, which make them suitable for use in antifungal, termiticidal, and repellent applications. In addition, wood vinegar exhibits a high degree of antimicrobial activity against various microorganisms (Ma et al., 2011).

Mangifera indica, *Tamarindus indica* and *Azadirachta indica* are widely cultivated in Thailand. To improve tree development and productivity in the following years, these tree must be pruned following each harvest, but most of the trimmings are discarded or burnt as fuel. However, these trimmings could be used to manufacture charcoal, producing wood vinegar as a byproduct. If the resulting wood vinegar possesses active biological properties, it would greatly increase the value of these tree cultivation. In addition, these bark have been an important herb in the medical systems. Various parts of plant are used as traditional medicine. Tamarind bark has been treated for abdominal pain, could well refer to diarrhea (Doughari, 2006).

Therefore, feeding of *Mangifera indica*, *Tamarindus indica* and *Azadirachta indica* wood vinegars to piglets may promote acidity in intestine, resulting in inhibition the growth of pathogenic bacteria and improve their performance. However, there are still few studies to test the effects of feeding wood vinegars in weaning piglets on antibacterial activity and growth performance.

1.2 Research Objectives

1.2.1 To examine composition of the wood vinegars from *Tamarindus indica, Mangifera indica, Azadirachta indica.*

1.2.2 To study the antibacterial activities of the wood vinegars from *Tamarindus indica, Mangifera indica, Azadirachta indica*.

1.2.3 To study the effect of supplementing wood vinegars from *Tamarindus indica*, *Mangifera indica*, *Azadirachta indica* on growth performance and health in weaned piglets.

1.3 Research hypothesis

1.3.1 The chemical profiles of *Tamarindus indica*, *Mangifera indica*, *Azadirachta indica* wood vinegars present different levels of bioactivity and different quantities of bioactive constituents.

1.3.2 *Tamarindus indica*, *Mangifera indica*, *Azadirachta indica* wood vinegars have potential as natural antibacterial agents and exhibits in vitro antibacterial properties against pathogenic bacteria.

1.3.3 The piglets fed the diet supplemented with *Tamarindus indica*, *Mangifera indica*, *Azadirachta indica* wood vinegars improved feed efficiency and health status.

1.4 Scope and limitation of the thesis

In the present study, *Tamarindus indica*, *Mangifera indica*, *Azadirachta indica* wood vinegar are represented for plant source of antibacterial agents. Wood vinegar in general has potential as a natural antioxidant and antimicrobial agent, many wood vinegar produced using different source materials might present different levels of bioactivity and different quantities of bioactive constituents. Gas chromatography mass spectroscopy (GC-MS), used to analyze, identify, and quantify the chemical compositions of wood vinegars. Wood vinegars exhibits a potential of antibacterial activity against pathogenic microorganisms. Therefore, wood vinegars are affordable and commercially available for using in animal feed as natural antibacterial agents. Weaned piglets from farm of Suranaree University of Technology were used in the studied of feed efficacy and blood parameter profiles. Piglets raised in evaporative cooling system houses so that housing climate and other factors can be controlled on condition.

1.5 Expected results

1.5.1 To expected results of the chemical profiles of *Tamarindus indica*, *Mangifera indica*, *Azadirachta indica* wood vinegars, different source materials might present different levels of bioactivity and different quantities of bioactive constituents.

1.5.2 To expected results of the effects of *Tamarindus indica*, *Mangifera indica*, *Azadirachta indica* wood vinegars on pathogenic bacteria, have been recognized as safe, natural various bioactivities, which make them suitable for use in antibacterial applications.

1.5.3 To expected results of the effects of feeding *Tamarindus indica*, *Mangifera indica*, *Azadirachta indica* wood vinegars on growth performance and health of weaned piglets.

1.5.4 This knowledge will be a valuable products for farmer and will enable it to use a suitable formulation.

1.5.5 Further research can use this knowledge base to create additional studies, they can be developed into useful sterile products for medical, aquaculture, and livestock breeding applications.

1.6 References

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CHAPTER II

LITERATURE REVIEW

2.1 Gastrointestinal health and functionality of pigs

Gastrointestinal tract (GIT) health is a term used very commonly, and is the subject of enormous interest currently throughout the world, yet generally lacks a precise and unifying meaning. Optimal gastrointestinal functionality is essential for sustainable animal production. Effective functionality of the GIT and its health are important factors in determining animal performance (growth, milk yield, meat and egg quality). Over the last few decades, the adoption of genetic selection for high growth and reproductive traits, the implementation of advanced husbandry techniques (hygiene, vaccination, housing, transport, etc.), improved understanding in digestive physiology and dietary requirements of farmed animals has led to significant gains in productive performance. It is within the concept of gut health has started to attract significant interest within the animal science community (Kogut and Arsenault, 2016)

The gastrointestinal tract is a multi-function organ, maintaining regular nutrient, water, and electrolyte absorption while acting as a barrier to exclude pathogens and toxins. The function of the mouth is chiefly to ingest feed by mastication, to lubricate it for the process of swallowing and contribute a regulation of the pH of the ingested feed by the saliva, Its selectively inhibit the binding of food to the teeth and mouth cavity, thereby minimizing agglutination and adherence of bacteria to the teeth (Pusztai et al., 1990). The stomach secreted the gastric mucosa are

HCl and pepsin, which are hydrolyzed protein into polypeptides. In pigs, carbohydrates are fermented into lactic acid and volatile fatty acids by gastric microbial fermentation. The stomach emptying in the pig is brought about by contractions passing down the pyloric region into the duodenum. Factors that trigger gastric emptying are the volume of its content and dietary composition. The rate of ingesta passage through the small intestine is very fast, regularly it takes 2.5 hours for a given particle to pass the small intestine. At this high speed of digesta passage it would be difficult for microorganisms to colonize this region. The epithelial cell layer is continuously regenerating, therefore microorganisms can colonize the small intestine if their proliferation rate is faster than the sloughing rate (Jonsson and Conway, 1992).

The small intestine is the primary site for absorption of amino acids, saccharides, lipids, vitamins and minerals. Bacteria are the most important of the symbiotic organisms breaking down carbohydrates and which predominantly occur in the hind gut of pigs. The environmental conditions in the hind gut for the host animal mainly from two aspects, including those for the microorganisms, with respect to an appropriate function of the processes in the hind gut, a certain amount of nutrients and bulk (indigestible organic matter) is needed, which has to be considered in the feeding strategy. Secondly, the feeding strategy has to take into account the enzymatic capacity of the animal, implying avoidance of overloading the digestive system and the transfer of unutilized nutrients from the small intestine into the distal part of the gastrointestinal tract (Thomke and Elwinger, 1998).

Bischoff (2011) further defined 5 major criteria that could form the basis of definition of gut health, with these being: 1) effective digestion and absorption of

food, 2) absence of GI illness, 3) normal and stable intestinal microbiome, 4) effective immune status, and 5) status of well-being. These definitions typically associate gut health with pathogens that cause, either clinically or sub clinically, illness, mortality and morbidity to pigs, and subsequent economic losses. In general agreement, the key components of GIT functionality are diet, effective structure and function of the gastrointestinal barrier, host interaction with the gastrointestinal microbiota, effective digestion and absorption of feed, and effective immune status. Nevertheless, the functions of the GIT extend beyond the processes associated with processes such as feed intake, digestion, and the subsequent active or passive absorption and barrier function, as the GIT plays a major role in regulating epithelial and immune functions of vital importance for normal biological functioning and homeostasis in both the GIT and the body (Celi et al., 2017).

2.1.1 The intestinal epithelium as a mucosal immunity

The intestinal epithelium is the major barrier that separates the external from the internal environment and represents the first line of defense against pathogens. They encircle the cells at the apical end of the lateral membrane and are composed of an array of proteins, including an integral membrane protein, the occludin, and cytosolic peripheral membrane proteins, the zonula occludens proteins (ZO-1, ZO-2). These proteins are in close apposition to the actin and myosin ring, in a dynamic adaptation to a variety of developmental, physiological and pathological circumstances (Sawada et al., 2003). The intestinal mucosa provided the largest immunological organs of the body which, through complex mechanisms, is able to remove pathogens, eliminate infected cells and develop an immunological memory to induce a more rapid response against a subsequent exposure to the same antigens. The

gut-associated lymphoid tissue (GALT) comprises follicles or groups of them defined as Peyer's patches, surrounded by specialised M cells, responsible for the transport in the patches of antigens and bacteria coming from the intestinal lumen (Stokes et al., 2004). The GALT also includes diffuse lymphoid tissue in the lamina propria, intraepithelial lymphocytes, mesenteric lymph nodes and an appendix. After antigen presentation by antigen presenting cells, lymphocytes leave the Peyer's patches, and migrate to effector sites, such as the spleen, lungs, respiratory and urogenital tracts. At intestinal sites, lymphocytes are spread in the lamina propria to produce antibodies (mainly IgA) or migrate to the epithelium. The intestinal immune system must protect the mucosa against pathogens, but to avoid hypersensitivity reactions against food proteins, normal microflora and innocuous macromolecules present in the intestine. It is known that reactions against food have an immunological basis and can derive from a breakdown of tolerance to microflora or food antigens (Strobel and Mowat, 1998). In addition, the mucosa is primed to launch transient inflammatory cascades upon colonization to promote immune responses, followed by desensitization to suppress excessive responses.Transient priming of the naive intestinal epithelial cells (IECs) may involve close contact with specific microbiota members, as demonstrated for segmented filamentous bacteria (SFB). In response to colonization, IECs enhance mucin and production of antimicrobial peptides (AMPs) to reduce microbial contact, whereas secretion of pro-inflammatory cytokines by IECs induces the development of effector T cells repertoires (Figure 2.1).

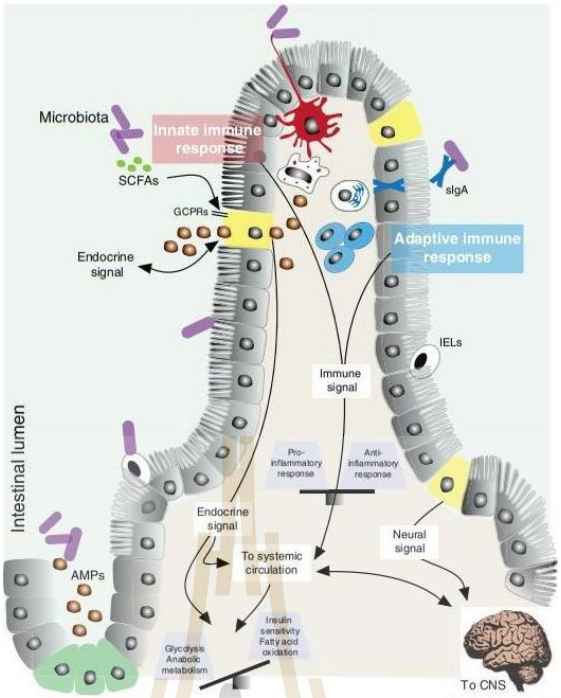

Figure 2.1 Multifactorial cross-talk between the microbiota and local and systemic host processes.

Source: Aidy et al. (2015).

In parallel, that drive multiple tolerance mechanisms, which for immune balance by suppressing excessive effector T-cell responses. Moreover, the development of immunoglobulin (IgA^+) producing plasma cells is induced to ensure an abundant supply of secretory (sIgA) that further limits microbial contact with the epithelium (Corthe, 2013). The tightly regulated immune development process in response to microbial colonization is essential to establish homeostatic coexistence and may be most amenable. Disruption of intestinal host microbe homeostasis can drive pro-inflammatory cascades, including the induction of IL-17 and IL-22 production, neutrophil recruitment and the expansion of effector T cell repertoires (TH1 and TH17) (Takatori et al., 2019) These responses can lead to intestinal

conditions that skew the microbiota composition and reduced abundance of more tolerogenic microbial groups, which can enhance inflammatory responses and promote IECs hyperproliferation.

2.1.2 Gastrointestinal tract microbiota

The intestinal microbiota represents a trade-off between helpful barrier functionality, synthesis of beneficial nutrients and proteins and improved energy harvest from dietary components with low inherent potential, and the deleterious effects of inflammation and sub-clinical and clinical pathologies. Elimination of the intestinal microbiome completely is not a realistic goal. Instead, focus should be on assisting the animal to regulate shifts in the intestinal microbiome such that rapid population swings are avoided and equilibrium is maintained. The succession pattern of microbial colonization of the gastrointestinal tract may have important immediate and long term implication for animal health and productivity. Petri and Co-authors (2010) studied to more closely establish microbial succession patterns throughout the GIT of the neonatal pig are presented in Table 2.1.

Throughout the host lifespan, complex-signaling and dynamic-signaling interactions shape the interplay between host and microbiota (Kostic et al., 2013) Exposure of the newborn to the maternal microbiota initiates the intestinal colonization by the microbiota of which the composition varies dramatically during the first year of life, after which it stabilizes to establish a complex and host-specific microbial community. The microbiota drives rapid maturation of the neonatal immune system, initially by activating innate immune functions followed by development of the adaptive immune system. The intestinal microflora helps the host to fight the colonization of pathogenic bacteria and to protect against dangerous substances

arriving in the colon. During life, microflora composition can transiently change in response to diet, health conditions and the environment (Akkermans et al., 2003).

Table 2.1 Bacterial family of 16s rRNA gene sequences from three GIT locations (stomach, small intestine and hindgut) at collection age 0.25, 0.5, 1, 2, 3, 5, 10 and 20 days of age.

No.	Family	n	(%)
1	Lactobacillaceae	141	23.3
2	Clostridiaceae	103	17.1
3	Streptococcaceae	93	15.4
4	Enterobacteriaceae	36	6.0
5	Moraxellaceae	21	3.5
6	Peptostreptococcaceae	19	3.1
7	Ruminococcaceae	14	2.8
8	Pasteurellaceae	16	2.6
9	Veillonellaceae	13	2.2
10	Micrococcaceae	5	0.8
11	Bacteroidaceae	4	0.7
	Total identified species	604	100.0

Source : Petri et al. (2010)

In young animals, the microbiota composition and its diversity can be influenced by environmental and management factors and as the GIT microbiota composition develops over time it then reaches equilibrium in adult animals. While

high microbiota diversity has been linked to higher resilience in adult animals, low diversity has been associated with gut health problems. On the other hand, lower microbiota diversity in young animals seems to be beneficial for developing towards an adult status. Microbial colonization of the piglet gut begins immediately following birth. Initial colonization by *E. coli* and *Streptococcus* spp. creates an anaerobic environment for subsequent colonizers, *Bacteroides*, *Bifidobacterium*, *Clostridium*, and *Lactobacillus*. A known beneficial microbe, *Lactobacillus*, predominates the small intestine of piglets until weaning and is a major player in disease prevention (Konstantinov et al., 2006; Petriet al., 2010). Microflora contributes to intestinal protection against pathogens by competition for pathogen binding sites, regulation of immune response, competition for nutrients, production of antimicrobial substances, such as bacteriocins, generation of restrictive physiological conditions, such as acidic pH caused by *lactobacilli* fermentations and by short chain fatty acid (SCFA) production.

In the post-weaning period, The piglets change from sows'milk to a solid diet of different composition and form undeniably plays a major role in the predisposition to diarrhea after weaning, both of microbial and dietary origin. The potential impacts of enterotoxigenic *E. coli* (ETEC) strains associated with infections and disease, i.e., postweaning colibacillosis (or post-weaning diarrhoea; PWD) and the means to prevent or control it. Amongst the physiological and GIT factors impacted by the weaning transition, microbiota disruption in the GIT is likely a key influence leading to PWD. Most of the studies conducted during the weaning transition have reported a decrease in bacteria of the *Lactobacillus* spp. Group and a loss of microbial diversity, whereas *Clostridium* spp., *Prevotella* spp. or facultative anaerobes such as

Proteobacteriaceae, including *E. coli*, were positively impacted (Gresse et al., 2017). Furthermore, in-feed and (or) in-water antibiotics also cause differences in the GIT microbiota at weaning due to their wide spectrum of activity and thus their potential ability to kill or prevent the growth of both pathogenic and beneficial. The diversity of the microbiota may be even more decreased (Looft et al., 2012), which can increase opportunities for pathogenic microorganisms to colonize and trigger diseases (Fouhse et al., 2016). In this regard, antimicrobial resistance is also key to any discussions pertaining to the use of antibiotics.

2.1.3 Barrier properties of the GI immune system

The resident immune cells and related lymphoid structures in the gut constitute the largest immune organ in the body. Given the massive antigenic luminal environment and continual exposure to luminal products, the GI immune system is tightly regulated via a number of molecular mechanisms, to prevent excessive activation and inflammation in response to the continual exposure to highly antigenic and inflammatory substances. However, the GI immune system must also be able to rapidly and robustly respond to any breach in the epithelial barrier or in the event of a pathogenic/antigenic challenge to mobilize innate and adaptive immune responses, which is critical in preventing the systemic spread of infection and inflammation. In summary, a delicate balance between control and reactiveness of the GI immune system is critical for optimal GI health and disturbances in this balance is central to GI inflammatory disorders and disorders associated with immune suppression (Adam et al., 2017).

2.2 The role of intestinal dysfunction in early weaning pigs

The impact of weaning on gastrointestinal health and disease has long been known. The first three months of postnatal life represent a major maturational period of GI development in the pig. During this time, intestinal epithelial, immune and enteric nervous system (ENS) phenotype and function change dramatically as the neonate adapts to life in the extra-uterine environment.

2.2.1 The critical window of postnatal GI barrier development

Many developmental processes exhibit a high degree of plasticity during this time and thus perturbations occurring in this critical window can largely shape the long-term phenotype and GI function. The establishment of the epithelial barrier develops rapidly in postnatal life and is characterized by a rapid decline in intestinal permeability. The process is critical to prevent exposure of the immune system to new environmental antigens from food and the colonizing microbiota, which would otherwise trigger massive inflammation. The development of intestinal epithelial barrier function occurs within the first 2 to 3 weeks of postnatal life characterized by a decline in intestinal permeability (Mackey et al., 2016). The precise mechanisms driving early postnatal barrier development are not well-defined but are thought to be driven by several factors including inherent genetic programming, microbial colonization and colostrum and milk factors.

Birth and weaning represent a major challenge to the developing immune system as it must adapt to GI microbial colonization and milk and feed antigens. In addition to a rapid epithelial barrier establishment, additional exogenous and endogenous factors act to suppress immune activation. For example, milk-derived immunoglobulins (e.g., immunoglobulin A, IgA), maternal leukocytes, and milk

glycans can act to modulate and neutralize intestinal microbes. Additionally, mothers milk provides anti-inflammatory cytokines and peptides, which suppress neonatal toll like receptor (TLR) and inflammatory cytokine expression (Newburg and Walker, 2007). In commercial pig production, weaning is abrupt occurring between 14 and 30 days of age. Whereas maternal separation is a major stressor to the weaned pig, additional psychosocial and immunological stressors, compound the stress load during this time, including transportation, mixing, fighting and establishment of a new social hierarchy, vaccination, etc. The timing of commercial weaning also coincides with a period of declining passive immunity from sow milk contributing an additional challenge to the pig. Weaned pigs are able to survive and overcome the stress of weaning; however, it is important to recognize early weaning stressors occurs during the critical window of GI barrier development. Given the role of the cholinergic nervous system in modulating immune responses, secretory diarrhea and the epithelial barrier, there are potential implications for an upregulated cholinergic system in early weaned pigs as a pathogenic mechanism for increased disease susceptibility associated with life stressors such as weaning.

2.2.2 Mechanisms driving intestinal barrier dysfunction in weaned pigs

Weaning is a stressful event in pigs as evidenced by the activation of hypothalamic pituitary adrenal axis (HPA) and elevation of stress related mediators, including CRF and cortisol in the circulation. Activation of the HPA axis is a critical survival mechanism to respond to a stressor and return to homeostasis. Serum CRF levels were shown to be elevated following weaning for the first week post-weaning, which mirrored changes in intestinal transepithelial permeability and electrogeniion transport properties (determine by measurement of transepithelial Isc) in pigs weaned

at 19 d of age (Moeser et al., 2007). In addition weaning-associated changes in the expression of the CRF system, we have also demonstrated that the CRF system is upregulated in pigs under-going other stressors including *Salmonella typhimurium* challenge (Boyer et al., 2015) suggesting a potential common pathophysiologic mechanism underlying stress-induced gut dysfunction in pigs.

2.3 Improvement of intestinal microflora

The mammalian gastrointestinal tract is a home to a complex and diverse microbial community that profoundly influences health and disease. The health of organisms largely depends on the composition of the intestinal microflora. The intestinal microbiota is a complex ecosystem that is intimately connected with the biology of the host and contributes to its homeostasis and health (Clemente et al., 2014). Its composition and function can be beneficially influenced by many factors.

2.3.1 Probiotics

Probiotics are live microbial feed supplements which beneficially affect the host by improving its intestinal microbiocenosis. Probiotics are mainly active in the caudal segments of the ileum, in the caecum and the ascending colon. They influence the digestive process in the body by increasing the activity of microbial probiotic enzymes and the digestibility of food (Roselli et al., 2005). They stimulate the immune system and the regeneration of intestinal mucosa. They can increase in immunoglobulin a production and stimulate macrophages and natural killers cells (Matsuzaki and Chin, 2000).

2.3.2 Prebiotics

Prebiotics are dietary short-chain carbohydrates, which cannot be digested by pigs, but are specifically utilizable by intestinal microflora. They support the growth and activities of probiotic microorganisms in the gastrointestinal tract. Prebiotic supplementation of the diet influences volatile fatty acid content (VFA), branched-chain proportion, lactic acid concentrations and ammonia concentrations in the gut (Pie et al., 2007). They increased concentrations of short-chain fatty acids (SCFA) stimulate natural bacterial activity and proliferation of bifidobacteria and lactic acid bacteria. The production of butyrate which is a dominant energy source for enterocytes also increases (Houdijk et al., 2002).

2.3.3 Synbiotics

Preparations containing probiotics and prebiotics increase the passage of probiotic bacteria through the upper part of the intestine and help the colonization of local receptors in the intestine. Some studies have confirmed a significant synergistic growth-stimulating effect (Shim et al., 2005), reducing mortality and increasing the counts of species that are components of natural microfloras (Frece et al., 2009). Furthermore, they was found that symbiotic preparations increase SCFA production (Bird et al., 2009) and reduce faecal noxious gas emission (Lee et al., 2009).

2.3.4 Acidifier diets

The addition of acidifiers to weanling pig diets requires a clear understanding of the acidifiers mode of action. Certain researchers have proposed that adding dietary acidifiers to swine diets correlates primarily with (1) decreased pH in the stomach and lower GIT, (2) modulation of microbial populations, and (3)

improvement in nutrient digestion (Partanen and Mroz, 1999). The acids can penetrate the bacterial cell wall and disrupt the normal actions of certain types of bacteria including *Salmonella* spp., *E. coli*, *Clostridia* spp., *Listeria* spp. and some coliforms. Therefore, reduction in numbers of some species of the normal intestinal bacteria as well as pathogenic bacteria can occur in animals fed acids.

2.3.4.1 Response of weaned pigs to various acidifier

In the search for feed acidifier diet with gut pH stabilizing effects, organic as well as inorganic acids have been proposed and used. The activity of most common acids, as well as their beneficial effects. Organic acids (C1-C7) are widely distributed in nature as normal constituents of animal tissue. They are also formed through microbial fermentation of carbohydrates pre-dominantly in the large intestine of pigs. Many of them are also available as sodium, potassium or calcium salts. The advantage of salts over free acids is that they are generally odourless and easier to handle in the feed manufacturing process owing to their solid and less volatile form. They are also less corrosive and may be more soluble in water than the free acids (Partanen and Mroz, 1999).

Acetic acid, propionic and butyric acids: Acetic, propionic and butyric acids are the major end products of bacterial fermentation of dietary fiber in the colon of the pig and they are absorbed by passive diffusion. Absorption rate depends greatly upon the pKa and the luminal pH. When the luminal pH is below the pKa, short-chain fatty acids are absorbed very rapidly. Because the luminal pH in ileum, cecum and colon of the pig is generally over than 6.5, most short-chain fatty acids remain in their dissociated form and are poorly absorbed. However, Na-H ion exchange by the epithelial cells may decrease the local pH at the absorptive surface, leading to a shift

from the ionic to the free acid state, the absorption proceeds because of a concentration gradient between the lumen and epithelial cells (Chang and Rao, 1994). As a preservative, acetic acid inhibits the growth of many species of bacteria, and to a lesser extent of yeasts and moulds. The action of propionic acid is primarily against moulds. It has only poor activity against bacteria and none against yeasts, since the latter can metabolize it (Foegeding and Busta, 1991).

Fraise and co-authors (2013) demonstrated that acetic acid at concentrations as low as 0.166% has good activity against clinical and type strains of *Pseudomonas aeruginosa*, whereas 0.312% inhibits the meticillin-susceptible strain of *Staphylococcus aureus* (MSSA) and *Acinetobacter baumannii*. The key basic principle on the mode of action of acetic acid is that non dissociated (non ionized) can penetrate the bacteria cell wall and disrupt the normal physiology of certain types of bacteria that presented as PH-sensitive, meaning that they cannot tolerate a wide internal and external PH gradient (Patanen and Morz, 1999).

Citric acid*:* Citric acid is generally a less effective antimicrobial agent than other organic acids, partly because many microorganisms can metabolize it and also because of a low pKa (Foegeding and Busta, 1991). This is less anti-bacterial as compared to other acids. Adding 1.5 % citric acid to control diets did not significantly affect pH, the concentration of volatile (VFA) or non-volatile fatty acids (NVFA), or microflora (total anaerobes, *Lactobacilli*, *Clostridia*, *E. coli*) in the contents from the stomach, jejunum, cecum or lower colon of weanling pigs. Moreover, did not affect the severity or incidence of scouring after a post-weaning *E. coli* challenge (Risley et al., 1993).

***Formic acid**:* Formic acid is commonly used as a preservative in ensiling forage and various by-products which contain less substrate for the desirable production of lactic acid by lactobacilli. Eckel et al (1992) fed early weaned pigs with diets containing different levels of formic acid. Addition basal diet with 6, 12 and 18g kg-1 formic acid improved post-weaning growth by 23, 31 and 29%, respectively. At levels greater than 18 g kg-1, growth was depressed due to reduced feed intake. Formic acid is effective in small concentrations (21.7 mM) against *E. coli*. In addition, formic acid treatment can effectively eliminate *salmonella* from contaminated feeds (Frank, 1994).

Fumaric acid: Dietary fumarate are absorbed by a common Na+ -gradient mechanism which is specific for tri- and dicarboxylates (Wolffram et al., 1992). In the pig, fumaric acid arises as a metabolite from the degradation of phenylalanine and tyrosine and also occurs as an intermediate in the urea cycle and during purine synthesis. Sutton and Co-authors (1991) added 0.3 % Na-fumarate to a control diet, but did not see any significant effect of the acid on the concentration of SCFA and the density of *Lactobacilli* or *E. coli* along the GI-tract. The decreasing effect of 1 % fumaric acid on *E. coli* counts in the stomach of 8 week-old piglets, and an increasing effect on VFA in the cecum compared to a control diet. No effect on VFA concentration, *Lactobacilli* counts along the GI-tract or on *E. coli* in the duodenum, cecum, or colon was detected.

Lactic acids: Lactic acid is produced by many bacterial species, The antimicrobial action of lactic acid is directed primarily against bacteria. This acid is a natural constituent of some feed stuffs and also is produced by many bacteria like *Lactobacillus* spp., *Streptococcus* spp., *Bifidobacterium* spp. etc. The supplementation

of milk with 1 % lactic acid resulted in lower counts of coliform bacteria and *Lactobacilli* in the gastric and duodenum of 2 weeks-old weaned pigs as compared to normal milk. Piglets fed diets supplemented with 0.7, 1.4 or 2.8 % lactic acid also showed changes of gastrointesinal characteristics (Maribo et al., 2000). The pH in the gastrointesinal tract was reduced by the acid addition and the *Lactobacilli* density was reduced in the small intestine (1.4 % lactic acid) and higher in the caecum and colon (0.7 % lactic acid) of pigs fed the diet added lactic acid.

2.3.4.2 Growth performance and nutrient utilization

Acidifiers diets exerted a small, positive influence on the apparent total tract digestibility and retention of crude protein (CP) and energy, whereas the influences on other nutrients were found to be less consistent. A review reported by Partanen and Mroz (1999) also confirmed improvements in the total tract digestibility and retention of crude protein with acidification of diets for weaned piglets or fattening pigs. It is notable that the data we reviewed showed that acids clearly increase nutrient digestibility in the absence of a clear reduction in gastric pH. The effect of acids on gastrointestinal tract of nutrients and energy seems to depend on the type and level of acid applied. Metzler and co-authors (2007) reported different apparent total tract digestibility of crude protein and energy and on nitrogen (N) retention in pigs fed with various organic acids. Increased proportion of organic acids disturbs acid base balance, metabolic acidosis and decreased feed intake with a reduced performance. Formic acid affect to gastrointestinal tract of crude protein, whereas the influence on GI tract of organic matter and energy seems to be smaller. Generally, based on the available data, organic acids undoubtedly increase nutrient digestibility, especially crude protein digestibility. The response of digestibility to

acidifiers varies widely depending on diet ingredients and type and level of acid utilized.

The greatest improvement in performance is seen after weaning and the effect diminishes as the pigs age. The mean improvements of growth rate were 12.25% and 6.03% for 0-2 and 0-4 week post-weaning periods, respectively ($P<0.05$). Similarly, addition of acids to diets improved ($P<0.05$), though to a lesser degree, the performance of growing (3.51%) and finishing pigs (2.69%). Apparently, acidifiers significantly promote the performance of pigs. The beneficial effects of organic acids on growth performance have also lately been confirmed in several reviews (Risley and Kornegay, 1993; Partanen and Mroz, 1999). Summary of published data on effects of dietary acid supplementation on growth performance of weaned piglets have shown in Table 2.2

Table 2.2 Dietary acid supplementation on growth performance of weaned piglets.

Level of acid (g kg^{-1})	% changes in		
	ADG	**Feed intake**	**Gain:Feed**
[1]Citric acid 15%	+3.6	-3.0	+6.2*
[1]Fumaric acid 15%	+2.2	-3.1	+5.1
[2]Fumaric acid 20%	+10.7	+1.7	+7.4*
[2]Fumaric acid 30%	+7.6	-1.3	+7.4*
[3]Citric acid 15%	+11.4	+10.3	+0.8
[3]Citric acid 30%	+9.6	+10.3	-1.6
[3]Citric acid 50%	+1.7	+2.6	-3.1

* Significantly different from the control group (P<0.01)

Source: [1]Risley et al. (1991) [2]Giesting et al. (1991) [3]Johnson (1992)

2.4 Pyroligneous acid (wood vinegar)

Wood vinegar, pyroligneous acid or nam som kwan mai (Thai), are strong, smoke-flavored liquors obtained as by-products from the charcoal manufacturing process of wood (Mun et al., 2007). Wood vinegar, is a condensed acidic liquid obtained in the process of producing wood charcoal. It has a special smoky odor and its color is light-yellow to brown. Wood consists of three main components, cellulose, hemicelluloses and lignin. There are some variation in the relative abundance of these constituents in different species of wood but as rough guideline, cellulose is taken to be approximately 50% by dry weight of wood and the other two components contribute approximately 25% each to the dry weight of wood (Guillén and Manzanos, 2002). Wood vinegar is produced when smoke from charcoal production is

cooled by outside air while passing through a chimney or flue pipe. The cooling effect causes condensation of wood vinegar, particularly when the temperature of smoke produced by carbonization ranges between 80 and 180°C/176 and 356°F. The overall carbonization process gave charcoal in 20-32% and collected distillate in 30% compare to the weight of the fresh wood. The pH value of wood vinegar is low, ranging from 2–3. Chemically, wood vinegar consists of water, water-soluble organic compounds and water-insoluble organic compounds (Oasmaa and Peacocke, 2001). Wood vinegar contains a high number of compounds arising from wood carbohydrate thermal degradation such as aldehydes, ketones, diketones, esters, alcohols, acids, furan and pyran derivatives. In addition, it also contains a significant number of components arising from lignin thermal degradation such as phenol, guaicol, syringol, pyrocatechol and their derivatives, as well as a trace amount of some other components (Guillén and Manzanos, 2002). Organic components of some edible sources are presented in Table 2.3. Major component of wood vinegar products are organic acids such as acetic, formic, propioinic and phenol compounds, these have been reported antibacterial effect. The addition of organic acids like citric, fumaric, formic and propionic acid to the diets of pigs is one of the most widely used alternative for antibiotics and has been reported to improve their performance. Their effects have been related to reduction in the growth of coliform bacteria, known to be involved in digestive disorders (Partanen and Mroz, 1999).

Table 2.3 Organic components of wood vinegar (%).

Plant source	Acetic acid	Total Phenol	Methanol
Pinus sp.[1]	3.90	-	0.70
Quercus sp.[1]	8.00	-	0.90
Larix sp.[1]	3.90	-	22.40
Chamaecyparis[1]	3.60	-	1.00
Eucalyptus sp.[1]	7.80	-	0.00
Cryptomeria japonica [2]	4.59	1.62	-
Quercus serrata[2]	4.89	1.28	-
Pinus densiflora[2]	2.76	2.36	-
Bambusa sp.[3]	2.72	0.13	0.07
Hevea brasilliensis[4]	4.52	0.02	-

Source: [1]Yoshimoto (1994), [2]Yatagai et al. (2002), [3] Rattanavut et al. (2009), [4]Prasertsit et al. (2011)

2.4.1 Bio-efficacies of chemical components of wood vinegar

Acetic acid: Acetic acid has been commonly used as medicine for the disinfection of wounds and especially, as an antiseptic agent in the treatment and prophylaxis of the plaque. Entani and co-authers (1998) reported the vinegar had a bactericidal effect on food-borne pathogenic bacteria including enterohemorrhagic *E. coli* (EHEC) O157:H7. The growth of all strains evaluated was inhibited with 0.1% concentration of acetic acid in the vinegar. Various conditions for bactericidal effects on EHEC O157:H7 were examined by the multiparametric analysis of five factors: acetic acid concentration in the vinegar, sodium chloride concentration, temperature,

incubation time, and viable cell number. The combined use of vinegar and sodium chloride, with use of an appropriate treatment temperature, was found to be markedly effective for the prevention of bacterial food poisoning. The key basic principle on the mode of action of acetic acid is that non dissociated (non ionized) can penetrate the bacteria cell wall and disrupt the normal physiology of certain types of bacteria that presented as PH-sensitive, meaning that they cannot tolerate a wide internal and external PH gradient (Patanen and Morz, 1999). The acetic acid either chemically pure or as vinegar, had a marked influence on the growth of different types of bacteria. The same PH, greater inhibition was obtained with an increasing concentration of acetic acid (Beuchat and Golden, 1999). Fraise and co-authors (2013) demonstrated that acetic acid at concentrations as low as 0.166% has good activity against clinical and type strains of *Pseudomonas aeruginosa*, whereas 0.312% inhibits the meticillin-susceptible strain of *Staphylococcus aureus* (MSSA) and *Acinetobacter baumannii*.

Phenolic compounds: Phenolic compounds such as phenol, guaiacol and cresols were found to give antimicrobial, antioxidant and smoke flavor properties. Phenol which was obtained from coal tar has been widely used as a disinfectant for industrial and medical applications. Phenolics are membrane active antibacterial agents on the biochemical activities of bacterial cells. It was reported that an antimicrobial action of phenolic compounds was related to inactivation of cellular enzymes, which depended on the rate of penetration of the substance into the cell or caused by membrane permeability changes (Moreno et al., 2006). Increased membrane permeability is a major factor in the mechanism of antimicrobial action, where compounds may disrupt membranes and cause a loss of cellular integrity and eventual cell death. In general, phenolic compounds are poorly absorbed in the small

intestine; it is estimated that 90-95% of dietary phenolics accumulate in the colon (Clifford, 2004). In the gut, phenolics may selectively suppress or stimulate the growth of some components of intestinal microbiota consequently, they may influence bacterial population dynamics (Tzounis et al., 2009).

Other organic components: Butyrolactone, corylone and malton, organic compounds found in wood vinegar, were tested for antimicrobial properties. Three synthetic butyrolactone derivatives were assessed for antimicrobial activity against Gram-positive and Gram-negative bacteria and fungi as for seed germination and seedling growth (Cazar et al., 2005).

2.5 Application of wood vinegar in agriculture

Due to wood vinegar composed of more than 200 chemical substances, wood vinegar has been widely used as a biocide and pesticide in agriculture, including plant production and protection as well as its role in animal husbandry based on old traditions and knowledge of users and local producers. Since the components of wood vinegar were naturally occurring organic compound, therefore, it was highly suitable for use in organic and hydrophonic farming, as well as conventional farming. Wood vinegar has been widely used in agriculture and daily life in Japan. There were about $4x10^7$ L produced every year and over half of it was used in agriculture (Higashino et al., 2005) Field experiments conducted in China have shown that wood vinegar, made from biomass residues, can be used as a foliar fertilizer improving the yield and quality of celery (Wei et al., 2009). A mixture of charcoal (biochar) and pyroligneous acid has also shown to enhance soil fertility and the growth of bedding plants (Kadota et al., 2004). Bamboo vinegar is known to simulate plant growth depending on the

pyrolysis temperature. Treatments with bamboo vinegar have also demonstrated an increase in vegetable growth (Mu et al., 2004).

2.5.1 Fungicide

The wood vinegars produced from biomasses such as inner coconut shells, bamboo and Eucalyptus wood effectively controlled fungal growth. The antifungal efficiency of wood vinegars was reported to be strongly dependent on their phenolic compounds contents (Baimark et al., 2009). Velmurugan and co-authors (2009) have reported that wood vinegar made from bamboo and broad leaved trees were effective against sapstaining fungi at minimum concentrations (0.10-1.0%). Results revealed that compounds of Chikusaku-eki and Mokusaku-eki markedly inhibited fungal growth. The same products have been used as disinfection, antibacterial and deodorization materials in agriculture and horticulture. However, Chikusaku-eki is mainly used as an ointment for the treatment of scabies, eczema, atopic dermatitis and other skin diseases.

2.5.2 Repellent and insecticide

Wood vinegar has been widely used to repel insects from plants and households in Thailand. Wood vinegar exhibited high termiticidal activities against the Japanese termite *Reticulitermes speratus* that a substituent of phenol played an important role in termiticidal activity (Yatagai et al., 2002).

2.5.3 Wood Vinegar as feed stuff

It has been suggested that wood vinegar can improve the performance of weanling pigs by improving the nutrient digestibility and reducing harmful intestinal coliforms. The performance of pigs fed with wood vinegar was found to be better than those fed with organic acid (Choi et al., 2009). Mixture of charcoal and

wood vinegar was proved to be useful as an aqua feed (Yoo et al., 2007).

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CHAPTER III

CHARACTERIZATION OF *TAMARINDUS INDICA, MANGIFERA INDICA AND AZADIRACHTA INDICA* WOOD VINEGARS

3.1 Abstract

Recently, drug resistance due to the extensive abuse and over-use of antibiotics has become an increasingly serious problem, making the development of alternative antibiotics a very urgent issue. In this study, The chemical compositions of wood vinegar from *T. indica*, *M. indica* and *A. indica* were analyzed by gas chromatography-mass spectrometry (GC-MS). The wood vinegar were collected at temperature ranges. The results showed the presence of key bioactive compounds, especially acetic acids with a range of 18.6 to 29.45%. The key basic principle on the mode of action of acetic acid is that non dissociated can penetrate the bacteria cell wall and disrupt the normal physiology of certain types of bacteria that presented as PH-sensitive, meaning that they cannot tolerate a wide internal and external PH gradient. The others compositions of wood vinegar were phenols, ketones, ester, benzene and its derivatives, aldehydes, alcohols, and some sugar derivatives. These compounds exhibit a wide range of physiological properties, such as antimicrobial and antioxidant effects.

This study revealed that the wood vinegar from *T. indica*, *M. indica* and *A. indica* would be a highly beneficial antibacterial activity for managing various ailment and valuable to develop as alternative antibiotic.

3.2 Introduction

Wood vinegar is a by-product from charcoal production. It is a complex mixture of 80 to 90% water, and 10 to 20% organic compounds. It contains several phenolic compounds such as guaiacol and cresol, and organic acids like acetic, formic and propionic acids. The addition of organic acids like citric, fumaric, formic and propionic acid to the diets of pigs is one of the most widely used alternative for antibiotics and has been reported to improve their performance. Acetic acid was formed during the thermal decomposition of hemicelluloses and lignin. In the pyrolysis reactions of biomass, water was formed by dehydration; acetic acid came from the elimination of acetyl groups originally linked to the xylose units and lignin, while furfural was formed by dehydration of the xylose units (Gaugler and Grigsby, 2009). Wood vinegar compositions and yields depend on process conditions and compositions of starting material. The carbonization of many different types of wood can be used to produce various wood vinegars including Eucalyptus (Pimenta et al., 2000), Oak (Guillen and Manzanos, 2002), bamboo (Mu et al., 2004), mangrove (Loo et al., 2008) and coconut shell (Wititsiri, 2011). Many different sources of wood vinegar have been recognized as safe, natural inhibitors with various bioactivities, which exhibits a high degree of antimicrobial activity against various microorganisms, along with significant antioxidant activity.

Plant materials have long been used as traditional medicines for the treatment of a wide variety of ailments and diseases. Research on medicinal plant has increased recently all over the world. The pharmaceutical companies have a lot of research in developing natural products extracted from plants, to produce more cost effective remedies that are affordable to the population. The acceptance of traditional medicine as alternative form of health care and the development of microbial resistance to the available antibiotics has provided to search for new antimicrobial substances from various sources like medicinal plants (Buwa et al., 2006).

Mangifera indica L., commonly known as mango, *Tamarindus indica* L., also known as Tamarind tree and *Azadirachta indica* L., also known as neem. There are popular cultivation in Thailand. Various parts of plants are used as traditional medicine. To improve trees development and productivity in the following years, These trees must be trimmings could be used to manufacture charcoal, producing wood vinegar as a byproduct. If the resulting wood vinegar possesses active biological properties, it would greatly increase the value of the trees cultivation. The aim of this study was to determines the chemical composition of wood vinegar from *M. indica*, *T. indica* and *A. indica*, using GC-MS to probe the relationship between the bioactivity and the constituents for utilize correctly and safely and effectively.

3.3 Materials and methods

3.3.1 Plants Collection

The fully grown plants, *T. indica*, *M. indica* and *A. indica* were collected from Rajabhat Agricultural Training and Research Center, Nakhon

Ratchasima Rajabhat University, Thailand. Wood samples were collected at the same times. Cure woods that have heartwood and bark for 5-15 days.

3.3.2 Wood Vinegars Preparation

Wood vinegar samples were prepared according to protocol described by Agricultural Production Sciences Research and Development Office, The Thailand Department of Agriculture.

In this study, all wood vinegars were produced by a pyrolytic retort kiln (Made by Rajamangala University of Technology Isan) (Figure 3.1A). All wood vinegars were prepared under the same conditions. Approximately 3 Kg of each wood was loaded into the retort. Each wood sample was slowly heated up to 400°C, wood vinegar were collected at three time ranges: 0 to 60 mins, 60 to 90 and 90 to 120 mins until cannot collect fluid (Figure 3.1B). The raw wood vinegar was stored at room temperature in a closed glass container protected from light for at least three months, which resulted in a wood vinegar that was a pale brown liquid on top of heavy tar for use.

(A) (B)

Figure 3.1 Production of wood vinegar (A), wood vinegar (B).

3.3.3 Chemical Compositions of Wood Vinegars from GC-MS

All wood vinegars were analyzed on a GC–MS (GC7890A, MS 7000 GC-MS Triple Quad, Autosampler GC sampler 120). A 60-m DB-WAX capillary column (0.25 mm I.D., 0.25-μm film) was used. Temperatures were maintained as follows: injection port 250°C, oven programmed at 35°C for 5 min, then raised at 3 °C/min to 100°C, then raised at 5°C/min to 240°C and held for 20 min. MSD conditions were as follows: capillary direct interface temperature 250 °C;ionization energy 70 eV. Injection volume was 3 μL in split mode (split ratio 10:1). Helium was used as a carrier gas with 1 ml/min flow rate. A solvent delay of 3 min was maintained. Tuning was performed using the auto-tune feature with perfluorotributyl-amine. All data were obtained by collecting the full-scan mass spectra within the scan range of 30-400 amu.

3.4 Results

3.4.1 Botanical Classification of *T. indica, M. indica* and *A. indica*

Kingdom	Plantae	Plantae	Plantae
Division	Tracheophyta	Tracheophyta	Tracheophyta
Class	Magnoliopsida	Magnoliopsida	Magnoliopsida
Order	Fabales	Sapindales	Sapindales
Family	Fabaceae	Anacardiaceae	Meliaceae
Genus	Tamarindus L.	Mangifera L.	Azadirachta A.
Species	*Tamarindus indica* L.	*Mangifera indica* L.	*Azadirachta indica* A.

Tamarindus indica L. is a tropical evergreen tree native area throughout Africa and Southern Asia. Tamarind cultivation has spread around the world in tropical and subtropical zones. *T. indica* is used as a traditional medicine in most of the tropical countries.

Mangifera indica L. belong to genus Mangifera L. which consists of about 30 species of tropical fruiting trees. There are over a thousand mango varieties around the world. Mango is one of the most popular of all tropical fruits. In addition, mango parts are used as folk medicine, one of main organs used is the bark.

Azadirachta indica A. is one of two species in the genus Azadirachta. It is typically grown in tropical and semi-tropical regions. Various parts of the neem tree have been used as traditional medicine, especially for leaf, fruit neem oil from seed and bark.

3.4.2 Description of *T. indica*, *M. indica* and *A. indica* Wood vinegar

Wood vinegar is produced when smoke from charcoal production is cooled by outside air while passing through a chimney or flue pipe. The cooling effect causes condensation of wood vinegar, particularly when the temperature of smoke produced by carbonization ranges between 80 and 180°C/176 and 356°F. The overall carbonization process gave charcoal in 20-32% and collected distillate in 30% compare to the weight of the fresh wood.

Table 3.1 Yields and physicochemical properties of wood vinegar obtained at time ranges.

Wood vinegar	Time (min)	Temp. (°C)	Volume (ml)	Colour	pH
T. indica No.1	0-60	0-385	500	Black	3
T. indica No.2	60-90	385-393	300	Brown	4
M. indica No.1	0-60	0-280	550	Black	4
M. indica No.2	60-90	280-340	500	Brown to yellow	3
A. indica No.1	0-60	0-270	730	Black	4
A. indica No.2	60-90	270-376	500	Brown	3
A. indica No.3	90-120	376-349	150	Brown	3

3.4.3 GC-MS Analysis

The various chemical compositions of all wood vinegars were analyzed by GC-MS. The absorbance peaks of the chemical components from each wood vinegar were shown in Figure 3.2-3.8. Their mass spectra of constituents were identified and compared with the database of NIST MS 14.0 which matched greater than or equal to 90%. The GC-MS analysis of the wood vinegar revealed different components as listed in Table 3.2-3.8. The GC-MS analysis indicated that there were ketones, phenols, acids, ester, benzene and its derivatives, aldehydes, alcohol, and sugar derivative in wood vinegar from these tree (Figure 3.9). It was found that phenols derivatives and acids were the primary components of all wood vinegar. The concentration of main acids and phenols from wood vinegar were shown in Table 3.9.

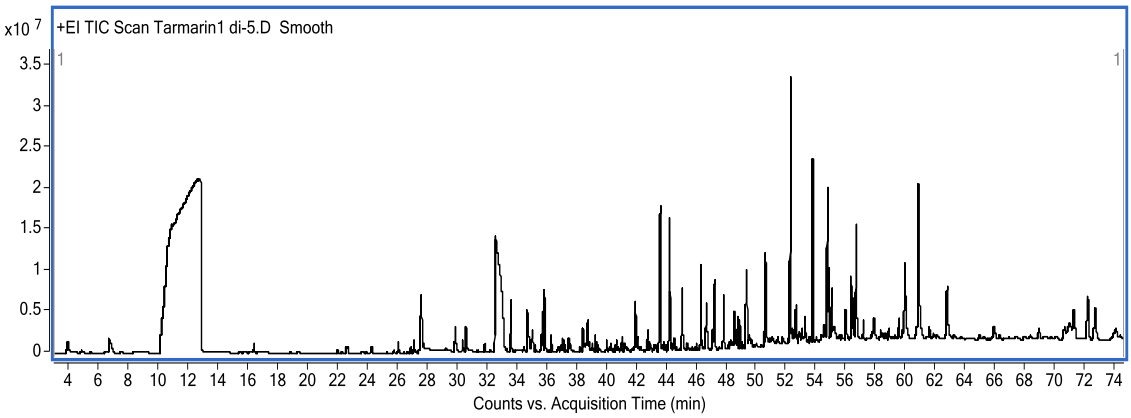

Figure 3.2 GC/MS chromatogram of *T. indica* No.1 wood vinegar.

Table 3.2 The chemical constituents of the *T. indica* No.1 wood vinegar analyzed by GC-MS.

No.	RT (min)	Compounds	Area percentage (%)
1	16.37	2-Ethoxytetrahydrofuran	0.3
2	24.23	Pyridine, 2-methyl-	0.2
3	26.01	Furan, 2,5-diethoxytetrahydro-	0.2
4	26.84	2-Butanone, 3-hydroxy	0.2
5	27.04	Furan, 2,5-diethoxytetrahydro-	0.3
6	27.55	2-Propanone, 1-hydroxy-	2.3
7	29.81	2-Cyclopenten-1-one	0.6
8	30.3	2-Cyclopenten-1-one, 2-methyl-	0.2
9	30.53	1-Hydroxy-2-butanone	0.6
10	32.46	Acetic acid	18.6
11	33.53	Furan, 2,5-dimethyl-	1.2
12	34.61	Formic acid	1.7

Table 3.2 (Continue).

No.	RT (min)	Compounds	Area percentage (%)
13	35.62	2-Cyclopenten-1-one, 3-methyl-	0.8
14	35.77	Propanoic acid	1.7
15	36.23	2-Cyclopenten-1-one, 2,3-dimethyl-	0.4
16	36.97	2-Furancarboxaldehyde, 5-methyl-	0.2
17	37.06	2-Pentanone, 5,5-diethoxy-	0.2
18	37.41	Pyridine, 3-methoxy-	0.2
19	38.35	Butanoic acid	0.5
20	38.6	1,2-Ethanediol, monoacetate	0.5
21	38.71	Butanoic acid, 4-hydroxy-	0.7
22	39.17	2-Furanone,2,5-dihydro-3,5-dimethyl	0.3
23	39.26	2-Furanmethanol	0.2
24	39.94	2(5H)-Furanone, 5-methyl-	0.3
25	40.96	2(5H)-Furanone, 3-methyl-	0.3
26	41.87	2(5H)-Furanone	1.1
27	42.68	2-Cyclopenten-1-one, 2-hydroxy-3,4-dimethyl-	0.4
28	43.52	1,2-Cyclopentanedione, 3-methyl-	3.8
29	44.15	Phenol, 2-methoxy-	2.8
30	44.98	2-Cyclopenten-1-one, 3-ethyl-2-hydroxy-	1.5

Table 3.2 (Continue).

No.	RT (min)	Compounds	Area percentage (%)
31	46.23	2-Methoxy-5-methylphenol	1.7
32	46.59	Maltol	0.9
33	47.05	Phenol, 2-methyl (o-Cresol)	0.3
34	47.15	Phenol	1.3
35	47.75	Phenol, 4-ethyl-2-methoxy-	1.1
36	48.48	2,2-Dimethyl-3-heptanone	0.9
37	48.69	p-Cresol	0.6
38	48.84	Phenol, 3-methyl-	0.6
39	49.31	Cyclopentanol	2.7
40	50.56	2(3H)-Furanone, dihydro-3-hydroxy-4,4-dimethyl-, (±)-	2.6
41	52.25	Phenol, 2,6-dimethoxy-	6.2
42	52.52	2(3H)-Furanone, 3-acetylihydro-3-methyl-	0.6
43	52.63	2,3-Anhydro-d-mannosan	0.7
44	53.20	Pentanoic acid, 4-oxo-	0.5
45	53.72	1,2,4-Trimethoxybenzene	3.9
46	54.70	5-tert-Butylpyrogallol	2.7
47	54.78	1,4:3,6-Dianhydro-α-d-glucopyranose	1.3
48	54.99	4(1H)-Pyridone	0.9
49	55.92	1-(2,5-Dimethoxyphenyl)-propanol	0.5

Table 3.2 (Continue.)

No.	RT (min)	Compounds	Area percentage (%)
50	56.29	S)-(+)-2',3'-Dideoxyribonolactone	1.6
51	56.39	5-Hydroxymethylfurfural	0.8
52	56.62	1,2-Benzenediol, 3-methoxy-	3.0
53	57.10	Phenol, 2,6-dimethoxy-4-(2-propenyl)-	0.4
54	57.82	Vanillin	0.4
55	59.48	Apocynin	0.5
56	59.88	2-Propanone, 1-(4-hydroxy-3-methoxyphenyl)-	3.1
57	60.78	Catechol	7.2
58	62.71	1,2-Benzenediol, 4-methyl-	2.3
59	65.85	1,3-Benzenediol, 4-ethyl-	0.7
60	68.85	Benzaldehyde, 4-hydroxy-3,5-dimethoxy-	0.4
61	70.55	Homovanillic acid	0.6
62	70.94	β-D-Glucopyranose, 1,6-anhydro-	1.4
63	71.18	Ethanone, 1-(4-hydroxy-3,5-dimethoxyphenyl)-	1.7
64	72.12	Desaspidinol	2.1
65	72.61	Hydroquinone	1.7

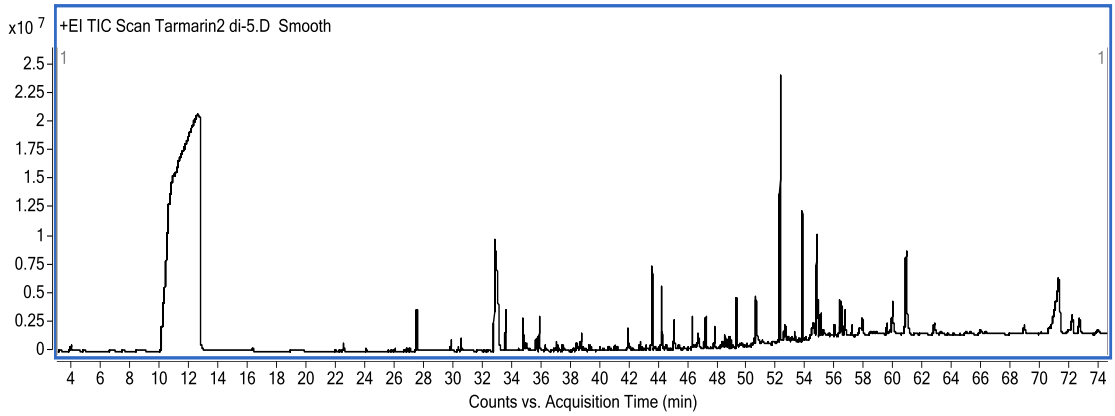

Figure 3.3 GC/MS chromatogram of *T. indica* No.2 wood vinegar.

Table 3.3 The chemical constituents of the *T. indica* No.2 wood vinegar analyzed by GC-MS.

No.	RT (min)	Compounds	Area percentage (%)
1	22.48	Pyridine	0.3
2	27.48	2-Propanone, 1-hydroxy-	2.2
3	29.81	Furan, 3-methyl-	0.5
4	30.49	1-Hydroxy-2-butanone	0.5
5	32.75	Acetic acid	19.6
6	33.52	Furfural	1.5
7	34.68	Formic acid	1.6
8	35.60	2-Cyclopenten-1-one, 3-methyl-	0.6
9	35.81	Propanoic acid	1.4
10	36.22	2-Cyclopenten-1-one, 2,3-dimethyl-	0.2

Table 3.3 (Continue).

No.	RT (min)	Compounds	Area percentage (%)
11	36.96	2-Furancarboxaldehyde, 5-methyl-	0.3
12	37.05	2-Pentanone, 5,5-diethoxy-	0.2
13	37.4	Pyridine, 3-methoxy-	0.2
14	38.96	Butanoic acid	0.3
15	38.68	Butanoic acid, 4-Hydroxy-	0.6
16	41.85	2(5H)-Furanone	1.0
17	43.50	1,2-Cyclopentanedione, 3-methyl-	3.3
18	44.15	Phenol, 2-methoxy-	2.5
19	44.98	2-Cyclopenten-1-one, 3-ethyl-2-hydroxy-	1.2
20	46.23	2-Methoxy-5-methylphenol	1.1
21	46.60	Maltol	0.6
22	47.15	Phenol	1.1
23	47.75	Phenol, 4-ethyl-2-methoxy-	0.9
24	48.46	2,2-Dimethyl-3-heptanone	0.5
25	48.68	p-Cresol	0.3
26	48.84	Phenol, 3-methyl-	0.5
27	49.24	Cyclopentanol	2.0
28	50.56	2(3H)-Furanone, dihydro-3-hydroxy-4,4-dimethyl-, (±)	2.4

Table 3.3 (Continue).

No.	RT (min)	Compounds	Area percentage (%)
29	52.22	Phenol, 2,6-dimethoxy-	10.9
30	52.51	2(3H)-Furanone, 3-acetyldihydro-3-methyl-	0.4
31	52.61	2,3-Anhydro-d-mannosan	0.4
32	53.21	Pentanoic acid, 4-oxo-	0.4
33	53.72	1,2,4-Trimethoxybenzene	4.8
34	54.69	5-tert-Butylpyrogallol	3.4
35	54.77	1,4:3,6-Dianhydro-α-d-glucopyronose	1.2
36	54.97	4(1H)-Pyridone	0.9
37	55.92	1-(2,5-Dimethoxyphenyl)-propanol	0.3
38	56.26	S)-(+)-2',3'-Dideoxyribonolactone	1.4
39	56.38	5-Hydroxymethylfurfural	1.2
40	56.62	1,2-Benzenediol, 3-methoxy-	1.3
41	57.10	Phenol, 2,6-dimethoxy-4-(2-propenyl)-	0.4
42	57.82	Vanillin	0.5
43	59.48	Apocynin	0.5
44	59.86	2-Propanone, 1-(4-hydroxy-3-methoxyphenyl)-	2.0
45	60.80	Catechol	6.2

Table 3.3 (Continue).

No.	RT (min)	Compounds	Area percentage (%)
46	62.72	1,2-Benzenediol, 4-methyl-	0.8
47	68.84	Benzaldehyde, 4-hydroxy-3,5-dimethoxy-	0.7
48	71.10	β-D-Glucopyranose, 1,6-anhydro-	7.2
49	71.17	Ethanone, 1-(4-hydroxy-3,5-dimethoxyphenyl)-	4.5
50	72.10	Desaspidinol	1.5
51	72.59	Hydroquinone	1.5

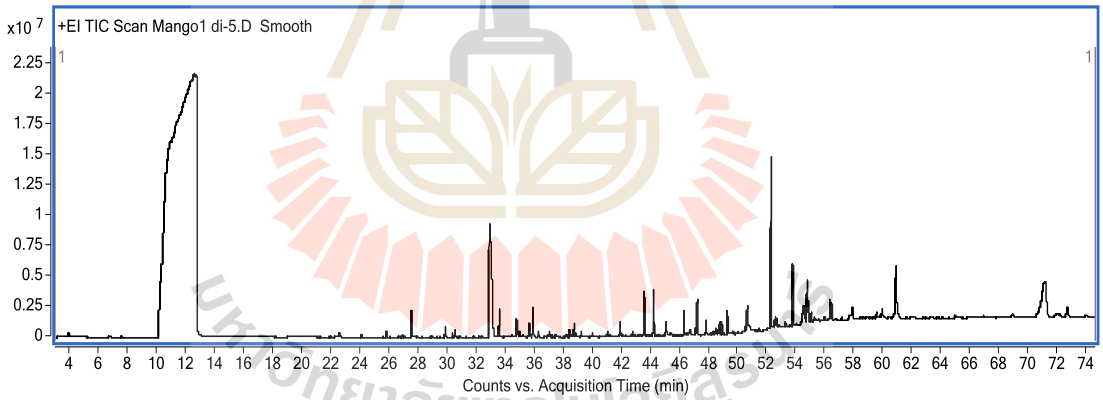

Figure 3.4 GC/MS chromatogram of *M. indica* No.1 wood vinegar.

Table 3.4 The chemical constituents of the *M. indica* No.1 wood vinegar analyzed by GC-MS.

No.	RT (min)	Compounds	Area percentage (%)
1	22.54	Pyridine	0.3
2	27.48	2-Propanone, 1-hydroxy-	2.1
3	29.82	Furan, 3-methyl-	0.7
4	30.50	1-Hydroxy-2-butanone	0.5
5	32.84	Acetic acid	25.3
6	33.32	Furfural	1.7
7	34.72	Formic acid	1.4
8	35.60	2-Cyclopenten-1-one, 3-methyl-	0.6
9	35.82	Propanoic acid	1.9
10	36.22	2-Cyclopenten-1-one, 2,3-dimethyl-	0.4
11	36.97	2-Furancarboxaldehyde, 5-methyl-	0.3
12	38.36	Butanoic acid	0.6
13	38.67	1,2-Ethanediol, monoaceate	0.8
14	39.17	Butanoic acid, 4-Hydroxy-	0.3
15	40.96	2-Furanone, 2,5-dihydro-3,5-dimethyl	0.3
16	41.85	2(5H)-Furanone	1.0

Table 3.4 (Continue).

No.	RT (min)	Compounds	Area percentage (%)
17	42.67	2-Cyclopenten-1-one, 2-hydroxy-3,4-dimethyl-	0.3
18	43.50	2-Cyclopenten-1-one, 2-hydroxy-3-methyl-	2.9
19	44.15	Phenol, 2-methoxy-	2.9
20	44.98	2-Cyclopenten-1-one, 3-ethyl-2-hydroxy-	1.0
21	46.23	2-Methoxy-5-methylphenol	1.4
22	47.06	Phenol, 2-methyl (o-Cresol)	0.4
23	47.15	Phenol	2.0
24	47.75	Phenol, 4-ethyl-2-methoxy-	1.0
25	48.46	2,2-Dimethyl-3-heptanone	0.4
26	48.68	p-Cresol	0.6
27	48.84	Phenol, 3-methyl-	0.9
28	49.23	Cyclopentanol	1.5
29	50.58	2(3H)-Furanone, dihydro-3-hydroxy-4,4-dimethyl-, (±)-	1.6
30	52.22	Phenol, 2,6-dimethoxy-	10.2
31	52.50	2(3H)-Furanone, 3-acetyldihydro-3-methyl-	0.6
32	52.61	2,3-Anhydro-d-mannosan	0.5

Table 3.4 (Continue).

No.	RT (min)	Compounds	Area percentage (%)
33	53.23	Pentanoic acid, 4-oxo-	0.3
34	53.72	1,2,4-Trimethoxybenzene	3.6
35	54.69	5-tert-Butylpyrogallol	2.1
36	54.77	1,4:3,6-Dianhydro-α-d-glucopyranose	1.1
37	54.97	4(1H)-Pyridone	0.5
38	56.26	(S)-(+)-2',3'-Dideoxyribonolactone	1.3
39	56.38	5-Hydroxymethylfurfural	1.1
40	57.82	Vanillin	0.8
41	59.48	Apocynin	0.5
42	59.87	2-Propanone, 1-(4-hydroxy-3-methoxyphenyl)-	0.7
43	60.81	Catechol	5.7
44	71.04	β-D-Glucopyranose, 1,6-anhydro-	14.4
45	72.60	Hydroquinone	1.5

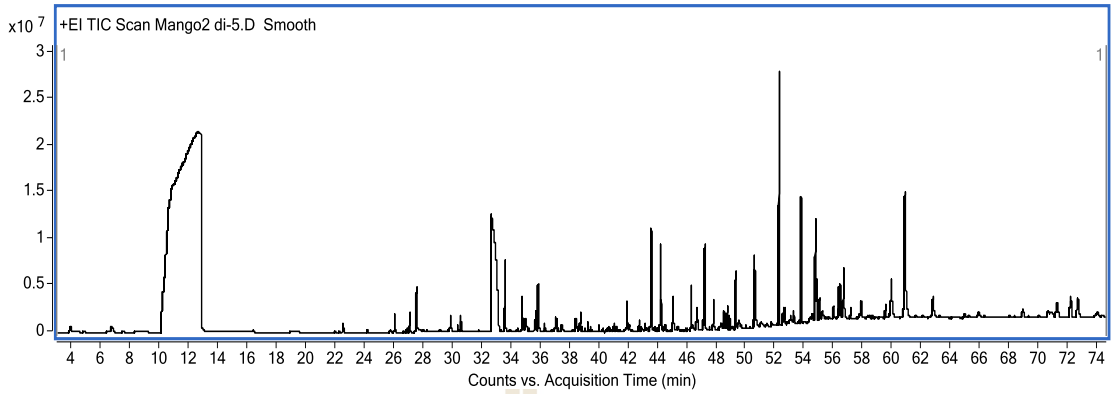

Figure 3.5 GC/MS chromatogram of *M. indica* No.2 wood vinegar.

Table 3.5 The chemical constituents of the *M. indica* No.2 wood vinegar analyzed by GC-MS.

No.	RT (min)	Compounds	Area percentage (%)
1	22.49	Pyridine	0.3
2	26.01	Furan, 2,5-diethoxytetrahydro-	0.7
3	27.04	Furan, 2,5-diethoxytetrahydro-	0.7
4	27.50	2-Propanone, 1-hydroxy-	2.0
5	29.81	Furan, 3-methyl-	0.7
6	30.51	1-Hydroxy-2-butanone	0.6
7	32.6	Acetic acid	23.8
8	33.53	Furfural	2.3
9	34.66	Formic acid	1.6
10	35.62	2-Cyclopenten-1-one, 3-methyl-	0.6
11	35.79	Propanoic acid	1.8

Table 3.5 (Continue).

No.	RT (min)	Compounds	Area percentage (%)
12	36.23	2-Cyclopenten-1-one, 3-methyl-	0.3
13	36.97	2-Furancarboxaldehyde, 5-methyl-	0.3
14	37.05	2-Pentanone, 5,5-diethoxy-	0.3
15	38.36	Butanoic acid	0.4
16	38.68	Butanoic acid, 4-hydroxy-	0.6
17	39.17	2-Furanone, 2,5-dihydro-3,5-dimethyl	0.3
18	41.86	2(5H)-Furanone	1.0
19	42.68	2-Cyclopenten-1-one, 2-hydroxy-3,4-dimethyl-	0.3
20	43.51	2-Cyclopenten-1-one, 2-hydroxy-3-methyl-	3.6
21	44.15	Phenol, 2-methoxy-	2.8
22	44.98	2-Cyclopenten-1-one, 3-ethyl-2-hydroxy-	1.3
23	46.23	2-Methoxy-5-methylphenol	1.2
24	46.59	Maltol	0.7
25	47.15	Phenol	2.6
26	47.75	Phenol, 4-ethyl-2-methoxy-	1.1

Table 3.5 (Continue).

No.	RT (min)	Compounds	Area percentage (%)
27	48.46	2,2-Dimethyl-3-heptanone	0.6
28	48.68	p-Cresol	0.8
29	48.84	Phenol, 3-methyl-	0.5
30	49.26	Cyclopentanol	2.3
31	50.55	2(3H)-Furanone, dihydro-3-hydroxy-4,4-dimethyl-, (±)-	3.1
32	52.23	Phenol, 2,6-dimethoxy-	8.8
33	52.51	2(3H)-Furanone, 3-acetyldihydro-3-methyl-	0.5
34	52.61	2,3-Anhydro-d-mannosan	0.4
35	53.21	Pentanoic acid, 4-oxo-	0.4
36	53.72	1,2,4-Trimethoxybenzene	3.9
37	54.70	5-tert-Butylpyrogallol	2.8
38	54.77	1,4:3,6-Dianhydro-α-d-glucopyranose	1.1
39	54.97	4(1H)-Pyridone	0.7
40	56.26	(S)-(+)-2',3'-Dideoxyribonolactone	1.0
41	56.39	5-Hydroxymethylfurfural	1.1
42	56.62	1,2-Benzenediol, 3-methoxy-	2.1
43	57.10	Phenol, 2,6-dimethoxy-4-(2-propenyl)-	0.3

Table 3.5 (Continue).

No.	RT (min)	Compounds	Area percentage (%)
44	57.82	Vanillin	0.5
45	59.48	Apocynin	0.5
46	59.87	2-Propanone, 1-(4-hydroxy-3-methoxyphenyl)-	2.2
47	60.79	Catechol	7.9
48	62.72	1,2-Benzenediol, 4-methyl-	1.3
49	68.84	Benzaldehyde, 4-hydroxy-3,5-dimethoxy-	0.6
50	71.18	Ethanone, 1-(4-hydroxy-3,5-dimethoxyphenyl)-	1.3
51	72.10	Desaspidinol	1.5
52	72.60	Hydroquinone	1.7

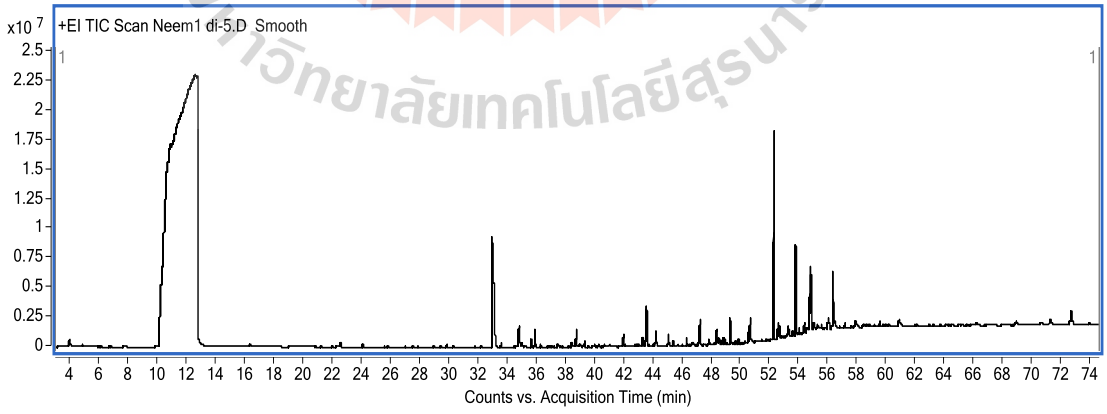

Figure 3.6 GC/MS chromatogram of *A. indica* No.1 wood vinegar.

Table 3.6 The chemical constituents of the *A. indica* No.1 wood vinegar analyzed by GC-MS.

No.	RT (min)	Compounds	Area percentage (%)
1	32.91	Acetic acid	24.8
2	34.75	Formic acid	2.0
3	35.59	2-Cyclopenten-1-one, 3-methyl-	0.6
4	35.83	Propanoic acid	1.5
5	38.37	Butanoic acid	0.4
6	38.68	Butanoic acid, 4-hydroxy-	1.4
7	41.85	2(5H)-Furanone	0.4
8	41.91	Acetamide	0.8
9	43.23	2H-Pyran-2-one, tetrahydro-	0.8
10	43.50	2-Cyclopenten-1-one, 2-hydroxy-3-methyl-	3.0
11	44.15	Phenol, 2-methoxy-	1.1
12	44.98	2-Cyclopenten-1-one, 3-ethyl-2-hydroxy-	1.2
13	45.33	2-Butenedioic acid (Z)-, dimethyl ester	0.5
14	46.23	Cresol	0.6
15	47.06	o-Cresol	0.3
16	47.15	Phenol	1.8
17	47.75	Phenol, 4-ethyl-2-methoxy-	0.6
18	48.29	2-Pyrrolidinone	1.4

Table 3.6 (Continue).

No.	RT (min)	Compounds	Area percentage (%)
19	48.46	2,2-Dimethyl-3-heptanone	0.6
20	48.68	p-Cresol	0.5
21	48.85	Phenol, 3-methyl-	0.7
22	49.23	Cyclopentanol	2.2
23	50.50	1,4:3,6-Dianhydro-α-d-glucopyranose	1.2
24	50.59	2(3H)-Furanone, dihydro-3-hydroxy-4,4-dimethyl-, (±)-	2.4
25	52.22	Phenol, 2,6-dimethoxy-	15.3
26	52.51	2(3H)-Furanone, 3-acetyldihydro-3-methyl-	1.5
27	52.61	2,3-Anhydro-d-mannosan	1.0
28	53.22	Pentanoic acid, 4-oxo-	1.0
29	53.53	Pyrrolidine, 1-(1-oxo-5-octadecenyl)-	0.5
30	53.72	1,2,4-Trimethoxybenzene	6.4
31	53.97	2(3H)-Furanone, 5-acetyldihydro-	0.5
32	54.32	Pyrrolidine, 1-acetyl-	1.0
33	54.7	5-tert-Butylpyrogallol	4.2
34	54.78	1,4:3,6-Dianhydro-α-d-glucopyranose	4.0

Table 3.6 (Continue).

No.	RT (min)	Compounds	Area percentage (%)
35	55.93	2,4-Hexadienedioic acid, 3,4-diethyl-, dimethyl ester, (Z,Z)-	0.4
36	55.99	Succinimide	0.8
37	56.27	(S)-(+)-2',3'-Dideoxyribonolactone	5.2
38	56.38	5-Hydroxymethylfurfural	0.7
39	57.10	Phenol, 2,6-dimethoxy-4-(2-propenyl)	0.4
40	57.83	Vanillin	0.6
41	60.82	Catechol	1.1
42	68.85	Benzaldehyde, 4-hydroxy-3,5-dimethoxy-	0.5
43	71.19	Ethanone, 1-(4-hydroxy-3,5-dimethoxyphenyl)-	1.1
44	72.61	Hydroquinone	2.9

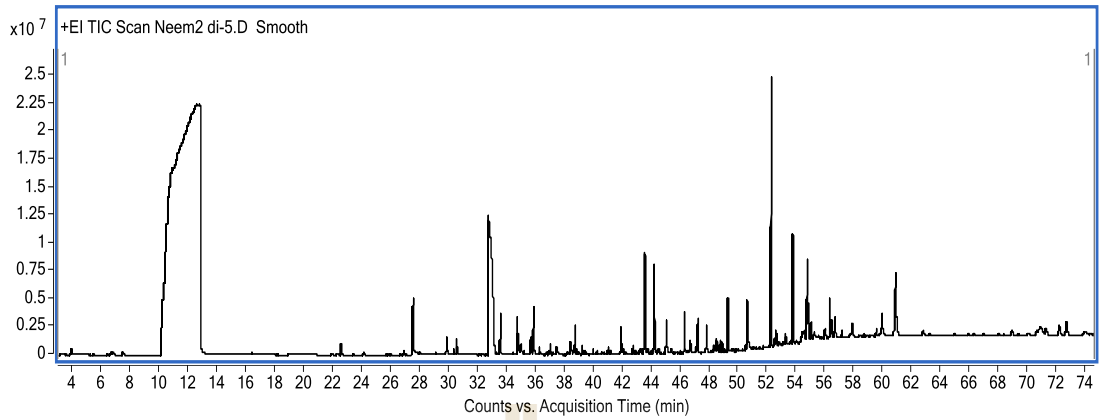

Figure 3.7 GC/MS chromatogram of *A. indica* No.2 wood vinegar.

Table 3.7 The chemical constituents of the *A. indica* No.2 wood vinegar analyzed by GC-MS.

No.	RT (min)	Compounds	Area percentage (%)
1	7.44	Sulfur dioxide	0.32
2	22.52	Pyridine	0.49
3	27.50	2-Propanone, 1-hydroxy-	3.29
4	29.82	2-Cyclopenten-1-one	0.85
5	30.51	1-Hydroxy-2-butanone	0.61
6	32.69	Acetic acid	29.45
7	33.53	Furfural	1.64
8	34.68	Formic acid	1.99
9	35.61	2-Cyclopenten-1-one, 3-methyl-	0.70
10	35.80	Propanoic acid	2.06
11	36.23	2-Cyclopenten-1-one, 2,3-dimethyl-	0.32

Table 3.7 (Continue).

No.	RT (min)	Compounds	Area percentage (%)
12	36.97	2-Furancarboxaldehyde, 5-methyl-	0.31
13	38.36	Butanoic acid	0.51
14	38.69	Butanoic acid, 4-hydroxy-	1.10
15	39.17	2-Furanone, 2,5-dihydro-3,5-dimethyl	0.27
16	39.94	2(5H)-Furanone, 5-methyl-	0.23
17	40.96	4-Hexen-3-one	0.26
18	41.13	1-Ethyl-3-methylcyclohexane (c,t)	0.24
19	41.85	2(5H)-Furanone	1.29
20	42.68	2-Cyclopenten-1-one, 2-hydroxy-3,4-dimethyl-	0.33
21	43.51	2-Cyclopenten-1-one, 2-hydroxy-3-methyl-	4.31
22	44.15	Phenol, 2-methoxy-	3.52
23	44.98	2-Cyclopenten-1-one, 3-ethyl-2-hydroxy-	1.44
24	46.23	2-Methoxy-5-methylphenol	1.47
25	47.06	Phenol, 2-methyl (o-Cresol)	0.22
26	47.15	Phenol	1.17
27	47.75	Phenol, 4-ethyl-2-methoxy-	1.07

Table 3.7 (Continue).

No.	RT (min)	Compounds	Area percentage (%)
28	48.29	2-Pyrrolidinone	0.24
29	48.46	2,2-Dimethyl-3-heptanone	0.49
30	48.69	p-Cresol	0.33
31	48.84	Phenol, 3-methyl-	0.48
32	49.24	Cyclopentanol	2.19
33	50.56	2(3H)-Furanone, dihydro-3-hydroxy-4,4-dimethyl-, (±)-	2.75
34	52.23	Phenol, 2,6-dimethoxy-	10.79
35	52.51	2(3H)-Furanone, 3-acetyldihydro-3-methyl-	0.55
36	52.61	2,3-Anhydro-d-mannosan	0.41
37	53.22	Pentanoic acid, 4-oxo-	0.41
38	53.72	1,2,4-Trimethoxybenzene	3.95
39	54.70	5-tert-Butylpyrogallol	2.68
40	54.78	1,4:3,6-Dianhydro-α-d-glucopyranose	1.24
41	54.97	4(1H)-Pyridone	0.79
42	55.19	Benzoic acid	0.45
43	56.27	(S)-(+)-2',3'-Dideoxyribonolactone	1.53
44	56.39	5-Hydroxymethylfurfural	0.58

Table 3.7 (Continue).

No.	RT (min)	Compounds	Area percentage (%)
45	56.63	1,2-Benzenediol, 3-methoxy-	1.01
46	57.10	Phenol, 2,6-dimethoxy-4-(2-propenyl)-	0.30
47	57.83	Vanillin	0.41
48	59.49	Apocynin	0.40
49	59.87	2-Propanone, 1-(4-hydroxy-3-methoxyphenyl)-	1.15
50	60.81	Catechol	4.30
51	62.73	1,2-Benzenediol, 4-methyl-	0.41
52	71.19	Ethanone, 1-(4-hydroxy-3,5-dimethoxyphenyl)-	0.65
53	72.12	Desaspidinol	0.81
54	72.61	Hydroquinone	1.24

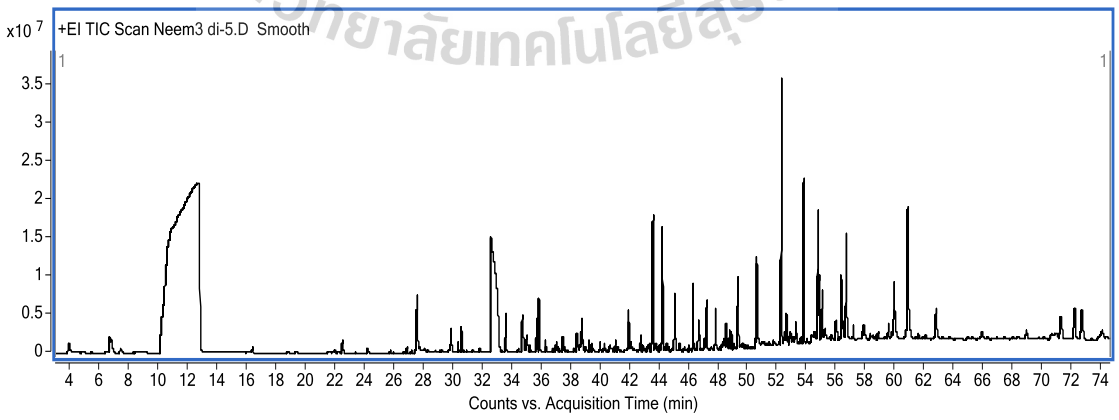

Figure 3.8 GC/MS chromatogram of *A. indica* No.3 wood vinegar.

Table 3.8 The chemical constituents of the *A. indica* No.3 wood vinegar analyzed by GC-MS.

No.	RT (min)	Compounds	Area percentage (%)
1	7.42	Sulfur dioxide	0.3
2	22.45	Pyridine	0.5
3	27.54	2-Propanone, 1-hydroxy-	3.0
4	29.81	Furan, 3-methyl-	0.8
5	30.52	1-Hydroxy-2-butanone	0.7
6	32.53	Acetic acid	20.9
7	33.53	Furfural	1.1
8	34.65	Formic acid	1.5
9	35.62	2-Cyclopenten-1-one, 3-methyl-	0.8
10	35.78	Propanoic acid	1.7
11	36.23	2-Cyclopenten-1-one, 2,3-dimethyl-	0.4
12	38.36	Butanoic acid	0.4
13	38.59	1,2-Ethanediol, monoacetate	0.3
14	38.72	Butanoic acid, 4-hydroxy-	0.9
15	39.17	2-Furanone, 2,5-dihydro-3,5-dimethyl	0.3
16	39.94	2(5H)-Furanone, 5-methyl-	0.2
17	40.96	4-Hexen-3-one	0.2
18	41.87	1-Ethyl-3-methylcyclohexane (c,t)	1.3

Table 3.8 (Continue).

No.	RT (min)	Compounds	Area percentage (%)
19	42.68	2(5H)-Furanone	0.4
20	43.52	2-Cyclopenten-1-one, 2-hydroxy-3-methyl-	4.3
21	44.16	Phenol, 2-methoxy-	3.3
22	44.99	2-Cyclopenten-1-one, 3-ethyl-2-hydroxy-	1.6
23	46.24	2-Methoxy-5-methylphenol	1.5
24	46.60	Maltol	0.7
25	47.06	Phenol, 2-methyl (o-Cresol)	0.2
26	47.15	Phenol	1.1
27	47.76	Phenol, 4-ethyl-2-methoxy-	1.1
28	48.32	2-Pyrrolidinone	0.2
29	48.48	2,2-Dimethyl-3-heptanone	0.7
30	48.69	p-Cresol	0.4
31	48.85	Phenol, 3-methyl-	0.5
32	49.30	Cyclopentanol	3.0
33	50.56	2(3H)-Furanone, dihydro-3-hydroxy-4,4-dimethyl-, (±)-	3.2
34	52.24	Phenol, 2,6-dimethoxy-	8.0
35	52.52	2(3H)-Furanone, 3-acetyldihydro-3-methyl-	0.6
36	52.62	2,3-Anhydro-d-mannosan	0.6

Table 3.8 (Continue).

No.	RT (min)	Compounds	Area percentage (%)
37	53.21	Pentanoic acid, 4-oxo-	0.5
38	53.73	1,2,4-Trimethoxybenzene	4.2
39	54.70	5-tert-Butylpyrogallol	2.7
40	54.78	1,4:3,6-Dianhydro-α-d-glucopyranose	1.4
41	54.99	4(1H)-Pyridone	1.3
42	56.29	(S)-(+)-2',3'-Dideoxyribonolactone	2.1
43	56.62	1,2-Benzenediol, 3-methoxy-	3.4
44	57.10	Phenol, 2,6-dimethoxy-4-(2-propenyl)-	0.3
45	57.83	Vanillin	0.3
46	59.49	Apocynin	0.5
47	59.88	2-Propanone, 1-(4-hydroxy-3-methoxyphenyl)-	2.5
48	60.79	Catechol	6.3
49	62.72	1,2-Benzenediol, 4-methyl-	1.4
50	65.85	1,3-Benzenediol, 4-ethyl-	0.4
51	68.85	Benzaldehyde, 4-hydroxy-3,5-dimethoxy-	0.4
52	71.19	Ethanone, 1-(4-hydroxy-3,5-dimethoxyphenyl)-	1.5

Table 3.8 (Continue).

No.	RT (min)	Compounds	Area percentage (%)
53	72.13	Desaspidinol	1.9
54	72.62	Hydroquinone	1.9

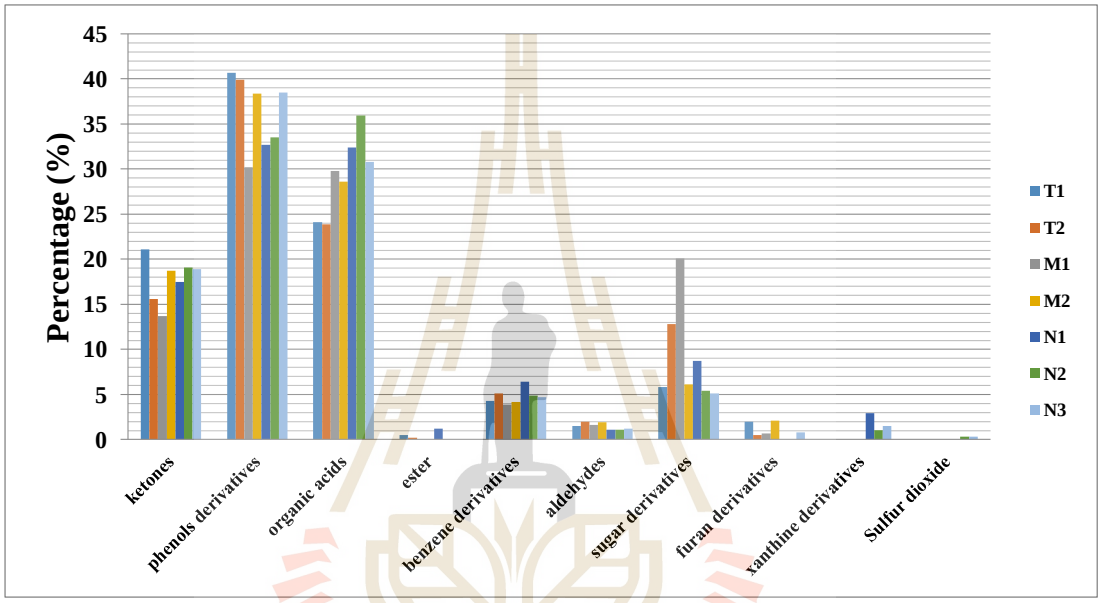

Figure 3.9 Chemical compositions of wood vinegar from *T. indica*, *M. indica* and *A. indica*.

Table 3.9 The concentration of main acids and phenols from wood vinegar.

Wood vinegar	Acetic acid	Formic acid	Propanoic acid	Butanoic acid	Phenol	p-Cresol
T. indica No.1	18.60%	1.70%	1.70%	0.50%	1.30%	0.60%
T. indica No.2	19.60%	1.60%	1.40%	0.30%	1.10%	0.30%
M. indica No.1	25.30%	1.40%	1.90%	0.60%	2.00%	0.60%
M. indica No.2	23.80%	1.60%	1.80%	0.40%	2.60%	0.80%
A. indica No.1	24.80%	2.00%	1.50%	0.40%	1.80%	0.50%
A. indica No.2	29.45%	1.99%	2.06%	0.51%	1.17%	0.33%
A.indica No.2	20.90%	1.50%	1.70%	0.40%	1.10%	0.40%

3.5 Discussion

Wood consists of three main components, cellulose, hemicelluloses and lignin. There are some variation in the relative abundance of these constituents in different species of wood but as rough guideline, cellulose is taken to be approximately 50% by dry weight of wood and the other two components contribute approximately 25% each to the dry weight of wood. Wood vinegar contains a high number of compounds arising from wood carbohydrate thermal degradation such as aldehydes, ketones, diketones, esters, alcohols, acids, furan and pyran derivatives. In addition, it also contains a significant number of components arising from lignin thermal degradation such as phenol, guaicol, syringol, pyrocatechol and their derivatives, as well as a trace amount of some other components (Guillén and Manzanos, 2002). These compounds exhibit a wide range of physiological properties, such as anti-inflammatory, antimicrobial and antioxidant effects.

In this study, The wood vinegar from *T. indica* (No.1 and No.2), from *M. indica* (No.1 and No.2) and *A. indica* (No.1, No.2 and No.3) were collected at the exit of a vapor funnel during the process of carbonization. The production of wood vinegar temperature range between 270°C to 393°C. Wood vinegars were collected at temperature ranges and were analyzed by GC-MS. *T. indica* and *M. indica* wood vinegar can collect within 90 min, *A. indica* wood vinegar within 120 min. The wood vinegars separated from the middle layer had smoke odor and transparent black to brown color and had distillation temperature at 70°C. The pH values of wood vinegars were 3-4. From sources of the raw materials of wood vinegar *T. indica*, *M. indica*, and *A. indica*, 800, 1050 and 1380 ml of wood vinegars were produced.

The compositions of wood vinegar were acids, phenols, ketones, ester, benzene and its derivatives, aldehydes, alcohols, and some sugar derivatives, Major component of wood vinegar products are acids such as acetic acid, formic acid, propanoic acid, butanoic acid and phenol compounds. These compounds were reported to shown the medicinal activity and physiological activity. The GC-MS analysis of these wood vinegars showed the presence of key bioactive compounds, especially acetic acids with a range of 18.6 to 29.45%., with concentration range 5,483.40 µg/ml to 88,065.10 µg/ml.

The acetic acid is subsequently purified and diluted with water to 60-80% by volume to obtain the ‘vinegar essence.’ In order to produce a food-grade vinegar, this ‘vinegar essence,’ which is a concentrated solution of acetic acid and highly corrosive, is further diluted until it contains 4-5% w/v of acetic acid. Many reviews have been reported antibacterial effect of acetic acids. The acetic acid had influence on the growth of different types of bacteria; *E.coli, Salmonella spp*. (Jensen et al., 2001).

Fraise and co-authors (2013) demonstrated that acetic acid at concentrations as low as 0.166% has good activity against clinical strains of *Pseudomonas aeruginosa*, whereas 0.312% inhibits *Staphylococcus aureus* (MSSA). The key basic principle on the mode of action of acetic acid is that non dissociated (non ionized) can penetrate the bacteria cell wall and disrupt the normal physiology of certain types of bacteria that presented as PH-sensitive, meaning that they cannot tolerate a wide internal and external PH gradient (Patanen and Morz, 1999). Phenolics are active antibacterial agents on the biochemical activities of bacterial cells. It was related to inactivation of cellular enzymes, which depended on the rate of penetration of the substance into the cell or caused by membrane permeability changes (Moreno et al., 2006). Increased membrane permeability is a major factor in the mechanism of antimicrobial action, where compounds may disrupt membranes and cause a loss of cellular integrity and eventual cell death. Based on this study, it is suggested that the wood vinegar from *T. indica*, *M. indica* and *A. indica* would be a highly beneficial antibacterial activity for managing various ailment and valuable to develop as alternative antibiotic. It can be noteworthy to explore these wood vinegar for further pharmacological interventions.

3.6 Conclusions

This is the first study of the chemical composition of wood vinegar from *Tamarindus indica, Mangifera indica and Azadirachta indica*. All wood vinegars were collected at the exit of a vapor funnel during the process of carbonization, with temperature range between 270°C to 393°C. The results reveal that wood vinegars showed the presence of key bioactive compounds, especially acetic acids with a range of 18.6 to 29.45%. The others composition of wood vinegar were phenols, ketones,

ester, benzene and its derivatives, aldehydes, alcohols, and some sugar derivatives. The chemical constituents of the wood vinegar must be identified to maximize utilization.

3.7 References

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CHAPTER IV

ANTIBACTERIAL ACTIVITIES OF *TAMARINDUS INDICA, MANGIFERA INDICA AND AZADIRACHTA INDICA* WOOD VINEGARS

4.1 Abstract

Wood vinegar is a condensed acidic liquid which obtained in the processing of wood charcoal production. In Thailand, it was used as a traditional medicine to control plant and animal diseases. In this study, three wood vinegars from each plant such as *Tamarindus Indica*, *Mangifera indica* and *Azadirachta indica* were evaluated the antibacterial activities using agar disc diffusion and broth microdilution methods, including the mode of action of each wood vinegars was determined by SEM. The results of antibacterial activity of the vinegar against Gram-positive and Gram-negative pathogenic bacteria was exhibited broad spectrum inhibition. The minimum inhibitory concentration (MIC) values were similar with ranging from 1.56-6.25 %v/v. The electron micrographs of cells treated with each wood vinegars showed the similar activity by the disrupted cell wall and membranes in Gram-negative bacteria. Microstructural observations showed that wood vinegars appeared depletion of the content of bacterial cells, indicating that the cell structures of treated bacteria were severely affected and damaged by the antibacterial agent. The treated cells showed an incomplete and deformed shape of the cells when compared with the untreated cells. There have been several reports that a pH value in culture condition may affect on the

conformation of the bacterial cell driven by the ATPase energy-consuming pump in bacterial cell membrane. Moreover, wood vinegar exhibited a pH in acidic value. The result suggested that wood vinegar may target on a bacterial cell membrane. Taken together, this research revealed that *Tamarindus Indica*, *Mangifera indica* and *Azadirachta indica* wood vinegars may be developed for an alternative antibacterial agent to control pathogenic bacteria.

4.2 Introduction

Recently, drug resistance due to the extensive abuse and over-use of antibiotics has become an increasingly serious problem, making the development of alternative antibiotics a very urgent issue. The incidence of multidrug resistance in pathogenic and opportunistic bacteria has been increasingly documented. The global search for alternatives to antibiotics. Plants remain the most common source of antimicrobial agents. Their usage as traditional health remedies is the most common in Asia, Latin America and Africa and are reported to have minimal side effects (Maghrani et al., 2005). The acceptance of traditional medicine as alternative form of health care and the development of microbial resistance to the available antibiotics has provided to search for new antimicrobial substances from various sources like medicinal plants (Buwa et al., 2006). *Mangifera indica*, *Tamarindus indica* and *Azadirachta indica* barks have been an important herb in the medical systems. The demonstration of antibacterial activity by the stem bark extracts of these plants against both gram positive and gram negative bacteria may be indicative of the presence of broad spectrum antibiotic compounds.

Wood vinegar is a condensed acidic liquid which obtained in the processing of wood charcoal production. The GC-MS analysis of the wood vinegar from *M. indica*, *T. indica* and *A. indica* indicated that there were ketones, phenols, acids, ester, benzene and its derivatives, aldehydes, alcohol, and sugar derivative in wood vinegar from these tree. It was found that acids were the primary components of all wood vinegar. Acetic acid inhibits the growth of many species of bacteria, the acids can penetrate the bacterial cell wall and disrupt the normal actions of certain types of bacteria including *Salmonella* spp., *E. coli*, *Clostridia* spp., *Listeria* spp. and some coliforms (Partanen and Mroz, 1999). Phenolics are membrane active antibacterial agents on the biochemical activities of bacterial cells. It was related to inactivation of cellular enzymes, which depended on the rate of penetration of the substance into the cell or caused by membrane permeability changes, where compounds may disrupt membranes and cause a loss of cellular integrity and eventual cell death (Moreno et al., 2006).

In this study, three wood vinegars from *T. indica*, *M. indica* and *A. indica* were evaluated the antibacterial activities using agar disc diffusion and broth microdilution methods, including the mode of action of each wood vinegars was determined by SEM, for utilize correctly and safely and effectively.

4.3 Materials and methods

4.3.1 Wood vinegar

The wood vinegar of *Tamarindus indica*, *Mangifera indica* and *Azadirachta indica* was collected and prepared by using a pyrolytic retort kiln (Produced by Rajamangala University of Technology Isan, Nakhon Ratchasima,

Thailand). The raw wood vinegar of *T. indica*, *M. indica* and *A. indica* in this experiments were selected from sample No.2 (collecting time 60-90 mins, pH 3-4) of all wood vinegar. This experiment assigned to four treatments (Enrofloxacin, *T. indica* wood vinegar, *M. indica* wood vinegar and *A. indica* wood vinegar).

4.3.2 Bacterial Strains and Culture Medium

Antibacterial activity was carried out against five selected pathogens (*Staphylococcus aureus* TISTR 746 *and Escherichia coli* TISTR 073). The strains used for present study were obtained from three sources. The strain of *Staphylococcus aureus* TISTR 746 *and Escherichia coli* TISTR 073 from The Thailand Institute of Scientific and Technological Research, Bangkok, Thailand. The strain of *Staphylococcus aureus* ATCC29213**,** Methicillin-resistant *Staphylococcus aureus* (MRSA) and *Escherichia coli* ATCC25922 that were used in this experiment, kindly gifted from Dr. Monton Visutthi, Department of Biology, Faculty of Science, Nakhon Ratchasima Rajabhat University.

Bacterial strains were isolated on Tryptic soy agar (Hi-Media, India) incubated at 37°C for overnight. The bacterial colonies were subcultured in Tryptic soy broth (Hi-Media, India) and then incubated at 37°C for 4-6 hrs. Mueller–Hinton (MH) medium (Hi-Media, India) was used for the agar diffusion method and minimal inhibitory concentration (MIC).

4.3.3 Agar Disc-diffusion Method

The antibacterial activities of bacterial strains on the different wood vinegar were determined using the disc-agar method described by the Clinical and Laboratory Standards Institute Standards. Briefly, sterile paper discs (6 mm in diameter) were loaded with 10 µL of wood vinegar (100% vv^{-1}) and then left to dry

for 18 hrs. at room temperature. The bacterial suspensions were then diluted to a turbidity of approximately 0.5 McFarland (~1.5×10^8 CFUmL^{-1}), and spread on MH agar with sterile cotton swab. Enrofloxacin (Susceptibility discs 5 µg/discs; Oxoid, UK) was used as the positive control. The plates were placed in an incubator at 37°C for 24 hrs. After incubation the antibacterial activity was determined by measuring diameter of the zone of inhibition around the discs. Each experiment was performed in triplicate and the results were expressed as means of four experiments.

4.3.4 Determination of minimal inhibitory concentrations (MICs)

The MIC values were used for determine bacteria which was found to be sensitive to wood vinegar. Susceptibility tests were conducted using the broth micro dilution method in according to the CLSI guidelines. Briefly, the MHB was then supplemented with serial dilutions of wood vinegar ranging from 0.39 to 25 % vv^{-1} in 96 well plate and as the control, enrofloxacin concentration was ranging from 0.03 to 250µg mL^{-1}. The bacterial suspensions were adjusted to a turbidity of 0.5 McFarland and then further diluted to obtain the final inoculum (1×10^6 CFUmL^{-1}) in MHB. After that an equal volume of 100 µL of the diluted inoculum was added to each well. After incubation at 37°C for 24 hrs. MIC values were recorded as the lowest concentration of the wood vinegar and antibiotic, when the bacteria do not exhibit visible growth, as indicated by the turbidity of the medium compared with the negative control. All assays were performed in triplicate.

4.3.5 Statistical Analysis

All data will be analyzed using SPSS. Differences between treatment means will be determine by Duncan's New Multiple Range Test. Differences among means with $P<0.05$ will be accepted as representing statistically significant differences.

4.3.6 Scanning Electron Microscope (SEM)

Escherichia coli TISTR 073 was used to study the effect of wood vinegar on morphological cell. After incubation with MIC level of wood vinegar for 0, 12, 24 and 36 hrs. The bacterial cells were harvested by centrifugation at 10,000 for 5 min. The cells were washed three times with 0.1 M Phosphate buffer (pH 7.4) and then fixed in 2.5% glutaraldehyde (Sigma, St. Louis, MO, USA) in PBS buffer at 4 °C for overnight. The bacterial cells were washed with PBS and then post-fixed in 4% osmium tetroxide (Sigma, St. Louis, USA) in PBS for 2 hrs. After washing with distilled water, the specimens were dehydrated in a graded acetone series (20-100%), mounted on aluminum stubs, allowed to dry and then catholic sprayed with gold. The samples were examined under a scanning electron microscope (Carl Zeiss, Auriga, Germany).

4.4 Results

4.4.1 Antibacterial activity

The results for the in vitro antibacterial properties of the wood vinegars are presented in Table 4.1. According to the agar disc-diffusion method, all of the test pathogens were sensitive to the wood vinegar with a range of the zone of inhibition diameter (ZI) of *S. aureus* TISTR 746 between 12.75-14.75 mm, *S. aureus* ATCC29213 between 7.45-9.93 mm, MRSA between7.23-7.75 mm, *E.coli* TISTR 073 between 10.38-11.88 mm, and *E.coli* ATCC25922 between 7.30-8.50 mm (Table 4.1), indicating that the wood vinegar possesses a broad antibacterial spectrum against different pathogens.

The results of minimum inhibitory concentration (MIC) determinations (Table 4.2) showed that the wood vinegar exhibits significant antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*, with respective MIC values of 1.56-6.25 %v/v.

Table 4.1 The zone of inhibition diameter (mm) of wood vinegars.

Item	***T.indica***	***M.indica***	***A.indica***	**Enrofloxacin**	***P*-value**
S. aureus TISTR 746	14.75±0.65^{b}	14.00±0.40^{b}	12.75±0.31^{b}	29.75±0.07^{a}	<0.01
S. aureus ATCC29213	8.90±0.58^{c}	9.93±0.15^{b}	7.45±0.52^{d}	28.38±0.25^{a}	<0.01
MRSA	7.75±0.29^{b}	7.68±0.10^{b}	7.23±0.15^{c}	11.68±0.39^{a}	<0.01
E.coli TISTR 073	11.88±0.68^{b}	11.50±0.17^{b}	10.38±0.32^{b}	30.00±0.05^{a}	<0.01
E.coli ATCC25922	8.00±1.47^{b}	8.50±1.56^{b}	7.30±0.69^{b}	29.38±1.31^{a}	<0.01

Values are shown as mean ± SD.

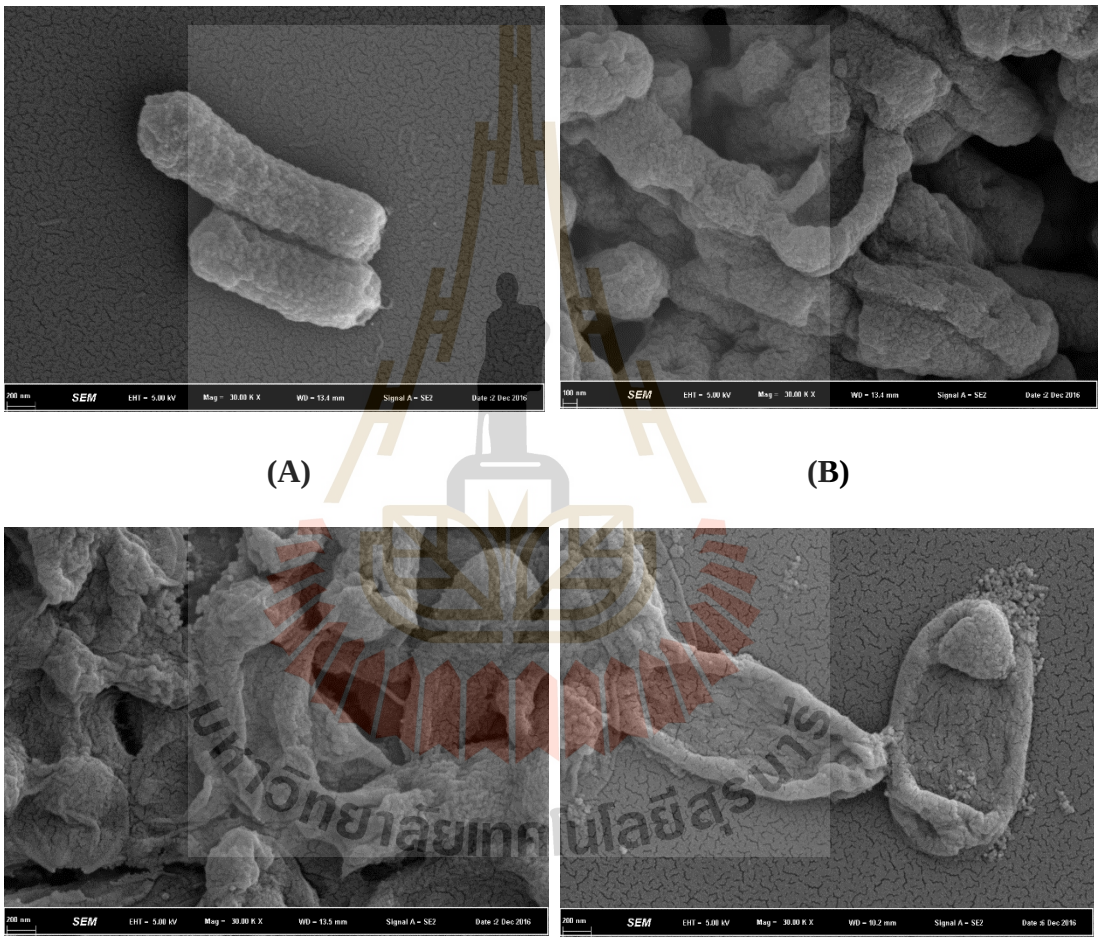
^{a,b,c,d} = Values in each row with different superscript were significantly differ. (P<0.05)

Table 4.2 The minimum inhibitory concentration (%v/v) of wood vinegars.

Item	***T.indica***	***M.indica***	***A.indica***
S. aureus TISTR 746	6.25	6.25	6.25
S. aureus ATCC29213	3.125	1.56	6.25
MRSA	3.125	1.56	6.25
E.coli TISTR 073	6.25	6.25	6.25
E.coli ATCC25922	3.125	1.56	6.25

4.4.2 Scanning Electron Microscope observation

To investigate the relationship between wood vinegar and bacterial cell membrane damage, The Scanning Electron Microscopy **(**SEM) studies were performed and the results revealed that the wood vinegars produced considerable morphological changes in the *E.coli* cells.



(A) **(B)**

(C) **(D)**

Figure 4.1 Scanning electron microscope (SEM) images of *E.coli* treated with *T. indica* wood vinegar at MIC levels (A) Untreated cells, (B) 12 hours, (C) 24 hours and (D) 36 hours of exposure to the wood vinegar.

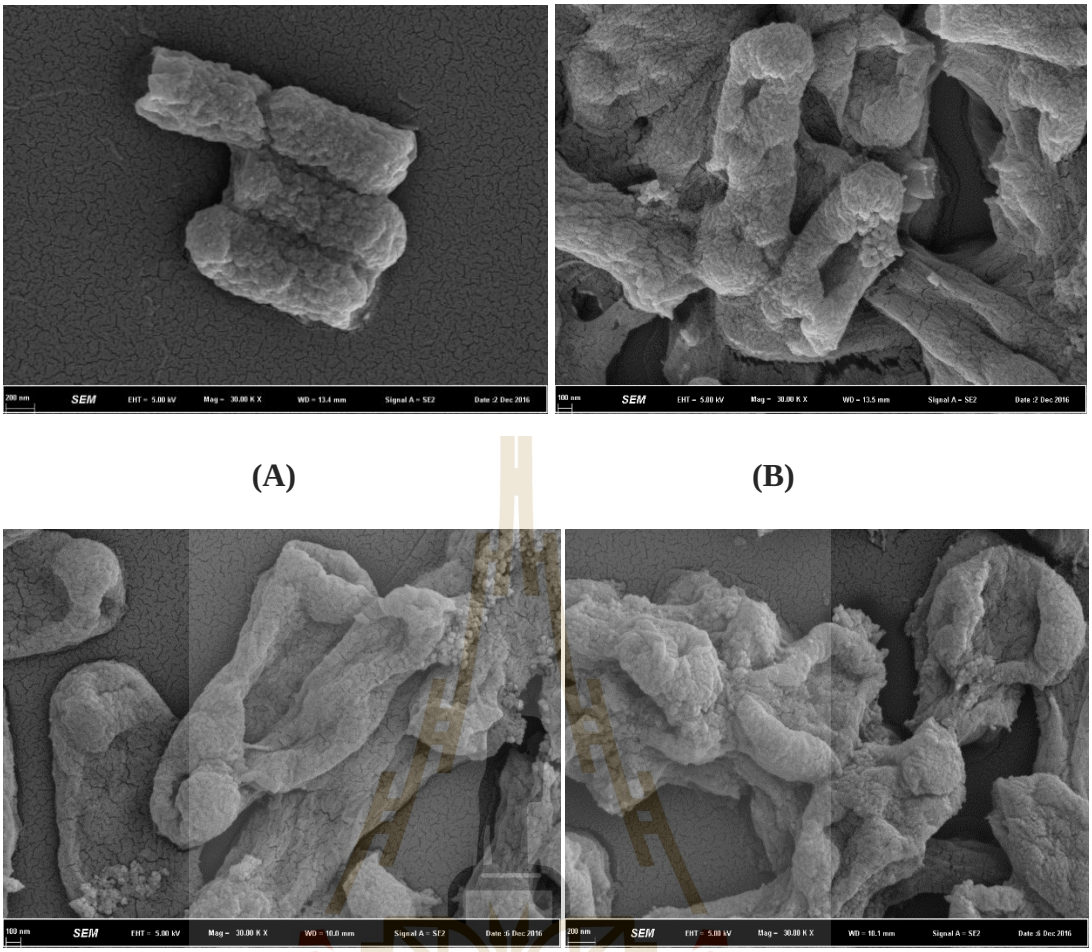
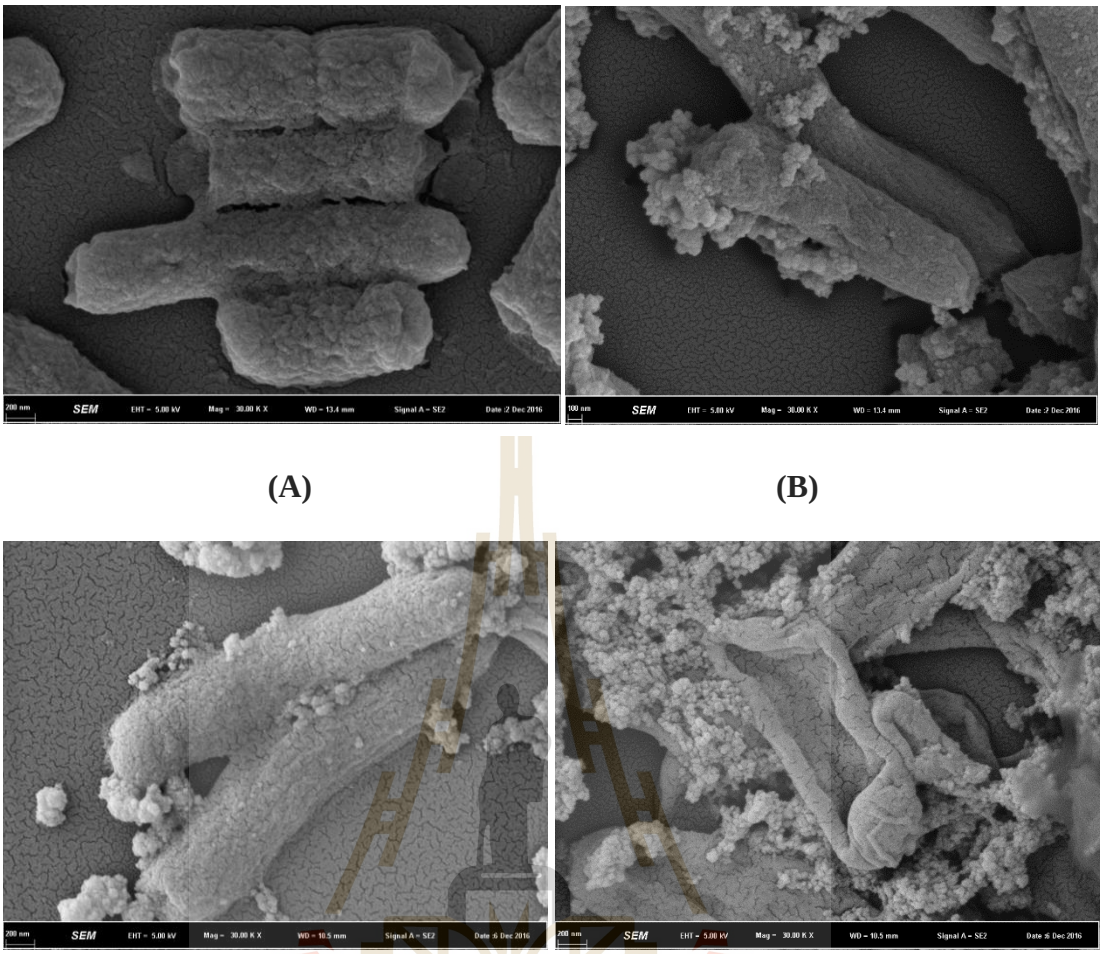


Figure 4.2 Scanning electron microscope (SEM) images of *E.coli* treated with *M. indica* wood vinegar at MIC levels (A) Untreated cells, (B) 12 hours, (C) 24 hours and (D) 36 hours of exposure to the wood vinegar.



(A) **(B)**

(C) **(D)**

Figure 4.3 Scanning electron microscope (SEM) images of *E. coli* treated with *A. indica* wood vinegar at MIC levels (A) Untreated cells, (B) 12 hours, (C) 24 hours and (D) 36 hours of exposure to the wood vinegar.

As shown in Figure 4.1A-4.3A, the results showed the SEM micrographs of the bacterial cells without wood vinegars treatment. It revealed the normal rod shape cell structure and complete surface. After 12 hour incubation with MIC dose of wood vinegar, the bacterial cells started to show multiple defects with many of cells exhibited crumpled or shrunken cell surface (Figure4.1B-4.2B). Figure 4.1C-4.2C revealed more formation of crumpled cells and some the cells formed cavities. After

36 hours of exposure (Figure 4.1D-4.2D), the bacterial cells were seemed to be totally deformed and collapsed cells were seen. Except in the *A. indica* wood vinegar (Figure 4.3B-4.3C), the cell morphology of bacteria was normal without any shrinkage or cavity formation as the surface was regular. However, After 12 hours treated with *A. indica* wood vinegar also showed some cell destruction and the cells were collapsed as compared to the control cells.

4.5 Discussion

The results of antibacterial activity of the vinegar against Gram-positive and Gram-negative pathogenic bacteria was exhibited broad spectrum inhibition. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration values were similar with ranging from 1.56-6.25 %v/v.

Other wood vinegar sources including bamboo, eucalyptus, and rubber also exhibited antimicrobial activity against dermatitis, environment, and plant bacteria, as well as fungi. A concentration of less than 10% is suggested as suitable for use as an antimicrobial agent (Chalermsan et al., 2009). Many previous reports suggest that Gram-positive bacteria are generally more susceptible to plant extracts than Gram-negative bacteria because of the absence of an outer membrane of lipoprotein and lipopolysaccharide. An outer membrane of lipoprotein and lipopolysaccharide is selectively permeable and can regulate access of antimicrobials into the underlying cell structures (Chan et al., 2012).

The electron micrographs of cells treated with each wood vinegars showed the similar activity by the disrupted cell wall and membranes in Gram-negative bacteria. Microstructural observations showed that wood vinegars appeared depletion of the

content of bacterial cells, indicating that the cell structures of treated bacteria were severely affected and damaged by the antibacterial agent. The treated cells showed an incomplete and deformed shape of the cells when compared with the untreated cells. There have been several reports that a pH value in culture condition may affect on the conformation of the bacterial cell driven by the ATPase energy-consuming pump in bacterial cell membrane (Chen et al., 1995; Lambert et al., 2001; Turgis et al., 2009). Moreover, wood vinegar exhibited a pH in acidic value. The result suggested that wood vinegar may target on a bacterial cell membrane. Taken together, this research revealed that *T. indica*, *M. indica* and *A. indica* wood vinegars may be developed for an alternative antibacterial agent to control pathogenic bacteria.

4.6 Conclusions

This is the first study of the antibacterial activities of wood vinegar from *Tamarindus indica*, *Mangifera indica* and *Azadirachta indica*. The wood vinegars showed significant antibacterial activities against clinical antibiotic-resistant pathogens, which implies they can be developed into useful sterile products for medical, aquaculture, and livestock breeding applications.

4.7 References

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CHAPTER V

THE EFFECT OF WOOD VINEGARS FROM *TAMARINDUS INDICA*, *MANGIFERA INDICA* AND *AZADIRACHTA INDICA* ON GROWTH PERFORMANCE AND HEALTH IN WEANED PIGLETS

5.1 Abstract

In this study, three wood vinegars from *T. indica*, *M. indica* and *A. indica* were investigations on growth performance, fecal bacterial population, hematology profile and intestinal villus morphology in weaned piglets. 120 weanling pigs (Duroc × Yorkshire × Landrace; 21±3 d of age) were randomly assigned according to receive 6 treatments. The treatments were control, antibiotic (enrofloxcacin), *T. indica* wood vinegar, *M. indica* wood vinegar, *A. indica* wood vinegar and acetic acid. No effects of wood vinegar and acid supplementation on growth performance. The piglets treated with *T. indica* and *M. indica* wood vinegar had reduced the fecal total bacteria concentration on day 28 relative to the control. The wood vinegars improved intestinal morphology by enhance villus height and VH:CD ratio in duodenum, jejunum and ileum except in *A. indica* wood vinegar was not different than control group. In the SEM observations, very large protuberated epithelial cells and microvilli were found on the duodenal and jejunal tip surface in the same treatment groups. Another important finding of the present study was the reduction in heart weight by treatment

with wood vinegar. The hematological traits and chemical profile of piglets fed the experimental treatments are normal ranges.

5.2 Introduction

Since their discovery, antibiotics have been used as therapeutic and growth promoting agents and this has lead to improvements in the performance of animals (Doyle, 2001). Antibiotic growth promoters have been widely used in the livestock industry due to their excellent health and growth promotion properties. There has been increasing concern that the use of antibiotics in food producing animals, particularly their long term use for growth promotion, contributes to the emergence of antibiotic resistant bacteria in animals. In the recent years, incidence of multidrug resistance in pathogenic and opportunistic bacteria has been increasingly documented (Jones et al., 2004). Organic acids, probiotics, prebiotics, and phytogenic substances have been tested as possible alternatives to replace antibiotics. The addition of organic acids to the diets of pigs is one of the most widely used alternative for antibiotics and has been reported to improve their performance. Their effects have been related to reduction in the growth of coliform bacteria, known to be involved in digestive disorders (Partanen and Mroz, 1999). Plant extracts and plant compounds are of new interest as antiseptics and antimicrobial agents, Although the active constituents may occur in lower concentrations, plant extracts may be a better source of antimicrobial compounds than synthetic drugs (Cox and Balick, 1994). Due to wood vinegar composed of many chemical substances, the main components were acids, therefore, wood vinegar could be applied in several fields. Wood vinegar has been widely used as a biocide and pesticide in agriculture, including plant production

and protection as well as its role in animal husbandry based on old traditions and knowledge of users and local producers.

The acetic acid can influence nutrient digestibility by modulating the balance of intestinal microflora and pathogens in ovariectomized rats (Kishi et al., 1999). Choi and co-authors (2009) reported that the inclusion of wood vinegar would increase the amino acid and energy digestibility of weaning pigs by modulating the intestinal microflora. Wood vinegar has been shown to induce a significant increase in egg production and improvements in the feed efficiency of laying hens (Li and Ryu, 2001). Nonetheless, there are fewer reports on the effect of wood vinegar in pigs and thus further scientific investigations are needed. In this study, three wood vinegars from *T. indica*, *M. indica* and *A. indica* were evaluated as an alternative to antibiotic for diarrhea prevention on bacterial communities, growth performance and blood characteristics in weaned piglets.

5.3 Materials and Methods

5.3.1 Experimental animals

All experimental procedures were conducted following the Ethical Principles and Guidelines for the Use of Animals issued by National Research Council of Thailand and approved by the ethics committee of Institute of Research and Development, Suranaree University of Technology. The study was conducted at a Suranaree University of Technology farm housing with an evaporative cooling system which maintained the temperature at 25°C and relative humidity below 80%.

In this study, 120 weanling pigs (Duroc × Yorkshire × Landrace; average body weight of 10.07 kg; 21±3 d of age) were randomly assigned according

to a Randomized Complete Block Design (RCBD) based on body weight and gender (male:female, 1:1) to receive 6 treatments. Each treatment had 4 replications with 5 pigs per replicate. The treatments were control, antibiotic (enrofloxcacin), *T. indica* wood vinegar, *M. indica* wood vinegar, *A. indica* wood vinegar and acetic acid. All treatments was administered orally every day at dose 0.5-1 ml./body weight 20 kg. Pigs were housed in partially slotted and concrete floor pens. The pigs in each pen received normal vaccine and disinfection procedures according to the pig farm's regulation, with a self feeder and nipple drinker to allow ad libitum access to the feed and water during 28 days of experimental period. All nutrient of basal diets exceeded NRC (1998) requirements. The initial and final body weights of pigs were recorded, respectively, and the corresponding feed consumptions were recorded per group in 28 days feeding periods. The daily feed intake, average daily gain and feed conversion ratio were calculated. At the end of the experiment, all the piglets were weighed and four piglets from each treatment were collect their blood samples and taken to a slaughterhouse; each piglet sedated and deep anaesthesia was applied by intravascular administrating 0.3 ml/kg of a mixture of Zoletil 100® (Virbac) and Xylazine hydrochioride (Xyl-M® 2%, VMD), followed by euthanized with a saturated injection of $MgSO_4$ solution into the jugular vein.

5.3.2 Blood sampling and analyses

Blood samples were collected from animals before and at the end of the experiment. Blood samples (3 ml) were collected from the jugular vein. The blood samples were collected using two types of tubes: the first tube was treated with ethylenediamine tetra-acetic acid (EDTA) to prevent blood coagulation, and the second tube was collected without EDTA. Whole blood samples from the first tube that contained EDTA were kept cold on ice at 4°C for hematological measurements.

The first tube was used for hematological analysis including red blood cells (RBC), hemoglobin, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelets, WBC count, and counts of neutrophils, lymphocytes, monocytes, eosinophils, and basophils. The second subsample was centrifuged (3000*g* for 15 minutes) within 30 minutes of collection, and Serum was frozen at -20 °C and later thawed for metabolite analysis (ALT, AST and Creatinine).

5.3.3 Fecal samples and bacterial counts

For analysis of fecal bacteria, Fecal samples were taken from six treatments randomly chosen pigs from each pen at 1, 7, 14, 21 and 28 days. Each fecal samples were collected by rectal swab technique (sterile cotton swab), placed in a sterile plastic container and stored at 4°C until analysis within each collection day. 10 g of fresh fecal sample was diluted with 9 ml 0.9% NaCl solution and then homogenized for 1 min and thereafter, One mL of fecal suspension was transferred into another tube containing 9 mL sterile 0.9% NaCl solution. A serial of 10-fold dilution was made to 10^{-3} to $^{10-8}$. Then, one mL of each dilution was duplicated and transferred to sterile agar plate and topped up with freshly made sterile agar and spread plate.

The culture media for total bacteria, *Escherichia coli* and *lactobacillus* counts, The total bacteria counts were determined using Nutrient Agar (HIMEDIA, India) incubated at 37°C for 24 hours; The *Escherichia coli* counts were determined using MacConkey agar (HIMEDIA, India) incubated at 37°C for 24 hours and for counting of *lactobacillus* bacteria, MRS agar (TM, India) was used and bacteria were grown in carbon dioxide incubator at 37°C for 48 hours, respectively. The dilution plates with colony numbers range of 30 to 300 colonies were recorded. Finally, an average of

duplicate plates was calculated and the bacterial population was transformed (log_{10}) before statistical analysis and expressed as log_{10} CFU/mL.

5.3.4 Tissue sampling

After the piglets were euthanized, Immediately after slaughtering, the abdominal cavities were opened along the midline. A mid-line incision was made to open the abdominal cavity and all the visceral organs were excised. Samples consisting of 5-cm sections of small intestine were taken at 20 cm from the stomach (regarded as the duodenum), in the middle of the small intestine (regarded as the middle part of the jejuno-ileum) and at 1 m from the ileo-caeco-colonic junction (regarded as the caudal part of the jejuno-ileum). In each intestinal segment, both ends of a 5 cm length were tied with a thread, flushed and kept in neutral buffered formalin solution and prepared for light and scanning electron microscopy.

5.3.5 Gross anatomical protocol

After removing the tissue samples for microscopic examination, the remaining small intestine was divided into gross anatomical segments based on surface structural features: Each part was cut, washed with 0.9% NaCl solution to remove the intestinal contents and measured for weight. Visceral organs such as the liver, heart, spleen, kidney, stomach and lung were also weighed.

5.3.6 Light microscopy

The samples were then cut into two parts from each segment for cross and length section of intestine surface. An 8×10-mm of each intestinal segment was cut from a 5 cm length of intestine, each segment was fixed in 10% formalin. Intestinal samples were excised and dehydrated for 16 h in tissue processing machine, Japan) then embedded in paraffin wax. Each section was cut 5 μm thicknesses and

fixed on to the glass slides, heated at 57°C until samples were dried. The samples were stained with haematoxylin and eosin, mounted with cover slips. The villus height was measured as the distance between the crypt mouth and the tip of villi. Three villi were selected under 10×10 magnification for each section. Thirty values of villus height were counted from 10 sections per piglet, and the mean villus height for each piglet was determined. The crypt depth was measured as the distance between the basement membrane and the mouth of crypt as using microscopes (Leica DM 750, Japan) and read by The I-views 2014 (Seek CO., LTD.). This procedure was based on the method described by Thu et al. (2011).

5.3.7 Scanning electron microscopy

A 2×3 cm segment was cut from the 5 cm duodenal segment close to the light microscopic sample and slit longitudinally along the non-mesenteric side for its entire length. The intestinal contents were washed with 0.1 M phosphate-buffered saline (pH 7.4). The tissue samples were pinned flat to prevent curling and fixed vertically with the mucosal surface facing downwards in the 10% buffer formalin at room temperature for 1 hr. The tissue block was further cut into a 3×10 mm rectangle and fixed for an additional 1 hr. The pieces were rinsed with Distilled water and then dehydrated with acetone. The specimens were dried in a critical point drying apparatus using liquid carbon dioxide as the medium. The dried specimens were coated with gold and observed with a scanning electron microscope (Carl Zeiss, Auriga, Germany). The morphological alterations of epithelial cells around the central apical surface of villi tips were compared between the groups.

5.3.8 Statistical Analysis

All the data measured in the feeding trial, fecal bacteria and in the LM examination were statistically analyzed using one-way analysis of variance, and the significant differences between the results of the treatments were determined with Duncan's multiple range test using the IBM SPSS Statistics program (Version 20.0; SPSS Inc, USA) at the $P<0.05$ level of significance.

5.4 Results

5.4.1 Growth performance of piglets

Overall effects of wood vinegar on piglets growth performances were determined. In Table 5.1, average piglets weight of all groups was 10.07±1.52 kg at the beginning of experiment.

On average, the final body weights of the control, antibiotic (enrofloxcacin), *T. indica* wood vinegar, *M. indica* wood vinegar, *A. indica* wood vinegar and acetic acid group were 25.05, 23.90, 21.00, 25.90, 22.65 and 24.85 kg, respectively. The body weight gains were 14.35, 14.02, 12.07, 14.87, 13.13 and 14.47 kg, respectively. The average daily gains were 512.50, 500.71, 431.07, 531.07, 468.93 and 516.79 g, respectively (Table 5.1). No statistically significant difference was found on growth performance ($P>0.05$).

Table 5.1 Growth performance of weaned piglets in different treatment groups.

Item	**control**	**antibiotic**	***T. indica***	***M. indica***	***A. indica***	**acetic acid**	***P*-value**
Initial body weight (kg)	10.70±0.37	9.88±2.06	8.93±1.45	11.03±0.74	9.52±0.58	10.38±1.76	0.27
Final body weight (kg)	25.05±1.30^{ab}	23.90±0.95^{bc}	21.00±0.82^{d}	25.90±0.53^{a}	22.65±0.81^{c}	24.85±1.38^{ab}	0.00
Body weight gain (kg)	14.35±1.61	14.02±2.10	12.07±1.19	14.87±1.03	13.13±1.16	14.47±1.60	0.14
Feed intake (kg)	24.82±0.79^{ab}	23.72±1.08^{abc}	21.30±1.91^{c}	26.19±2.34^{a}	22.28±0.32^{bc}	23.36±3.15^{abc}	0.02
ADFI (g/d)	886.57±28.25^{ab}	847.14±38.65^{abc}	760.54±68.34^{c}	935.18±83.72^{a}	795.54±11.28^{bc}	834.29±112.57^{abc}	0.02
ADG (g)	512.50±57.55	500.71±74.99	431.07±42.64	531.07±36.63	468.93±41.52	516.79±57.22	0.14
FCR	1.75±0.21	1.72±0.24	1.78±0.25	1.77±0.18	1.71±0.16	1.62±0.22	0.92

5.4.2 Hematology and chemical profile of piglets

The hematological traits and chemical profile of piglets fed the experimental treatments are shown in Table 5.2. At the starter phase, treatment did not differ significantly (P>0.05) among treatments in white blood cell (WBC) count, red blood cell (RBC) count, platelet (Plt) count, hemoglobin concentration (Hb), hematocrit (Hct), mean corpuscular volume (MCV) and white blood cell differential (WBC diff) count. There were however, significant differences (P<0.05) among treatments in mean corpuscular hemoglobin (MCH). Piglets fed the antibiotic had the lowest MCH. At the finisher phase, WBC count, RBC count, Plt count, Hb, Hct, MCV and WBC diff count values of piglets were not significantly (P>0.05) affected by treatments. There were significant differences (P<0.05) in Hb concentration between pre and post experiments. There were significant differences (P<0.05) among treatments in MCH values, however similar trend as was the case at the starter phase was also observed.

5.4.3 Fecal Bacterial Population

Effects of wood vinegar supplementation on fecal bacteria counts are shown in Table 5.3. Overall fecal bacterial population of treatment groups were similar with control, including *Escherichia coli* and *Lactobacillus* species. The average total bacteria in feces of control and treatment groups were $\log_{10}$ 2.15, 2.00, 2.05, 2.05, 2.12 and 2.07 CFU/mL, respectively. Only on day 28 of experiment, total fecal bacteria count of treatment (antibiotic, *T. indica* wood vinegar, *M. indica* wood vinegar, and acetic acid) group were decreased compared with the control group (P< 0.05). Similar circumstances to *Escherichia coli* bacteria, The average total *Escherichia coli* bacteria was $\log_{10}$ 1.97, 1.89, 1.94, 1.93, 1.95 and 1.94 CFU/mL. The treatment group appeared to be lower than control group during experiment periods, but did not

Table 5.2 Hematological and Biochemical Parameters of weaned piglets in different treatment groups.

Item		**control**	**antibiotic**	***T. indica***	***M. indica***	***A. indica***	**acetic acid**	**Reference range**
WBC. (Cell/Cu.mm)	Pre	17925±4791.23	16000±2943.92	20125±7054.73	14925±4158.02	17850±1011.60	19525±5725.60	11000-22000
	Post	19325±3155.29	16250±4255.59	16975±4804.43	20125±745.54	16675±3244.87	18875±2564.34	
Plt (Cell/Cu.mm)	Pre	268750±63331.80	353250±61716.42	401000±133947.75	357000±80651.51	393250±72862.31	308000±9237.60	200000-500000
	Post	362750±128549.28	328750±30280.63	284000±81030.86	378750±74593.90	350750±118094.24	433250±80875.93	
RBC. (X 10^6Cell/Cu.mm)	Pre	6.40±0.74	5.85±0.46	6.48±0.69	6.43±0.35	6.76±0.57	6.50±0.33	5-8
	Post	6.46±0.33	6.28±0.26	6.00±0.98	6.73±0.61	6.47±0.48	6.89±0.35	
Hb.(g/dl)	Pre	$12.45±1.28^{y}$	$11.63±1.00^{y}$	$12.83±1.57^{y}$	$12.53±0.66^{y}$	$13.28±1.42^{y}$	$12.40±0.64^{y}$	10-16
	Post	$13.38±0.39^{x}$	$13.13±0.82^{x}$	$13.20±0.75^{x}$	$13.35±1.12^{x}$	$12.70±1.16^{x}$	$13.73±1.06^{x}$	
Hct (%)	Pre	38.25±4.27	35.00±2.45	38.50±3.87	38.50±1.73	40.50±3.11	37.25±3.40	36-43
	Post	39.00±1.16	38.75±1.50	39.75±2.06	39.75±3.20	38.75±3.10	41.25±2.06	
MCV (fL)	Pre	52.00±1.83	50.75±2.06	57.25±1.26	55.00±5.83	51.75±1.00	51.00±1.41	50-68
	Post	51.50±1.73	53.75±5.12	52.00±2.45	52.75±3.10	51.25±2.22	51.00±0.82	
MCH (pg)	Pre	$20.00±0.82^{a}$	$18.00±1.41^{b}$	$21.25±0.96^{a}$	$19.50±0.58^{a}$	$19.50±0.58^{a}$	$19.25±0.96^{a}$	17-21
	Post	$19.75±1.26^{a}$	$19.00±0.82^{b}$	$20.00±1.41^{a}$	$20.75±1.71^{a}$	$20.25±0.96^{a}$	$20.25±1.26^{a}$	

Table 5.2 (Continue).

Item		control	antibiotic	*T. indica*	*M. indica*	*A. indica*	acetic acid	Reference range
MCHC (g/dl)	Pre	31.25±1.26	30.50±1.73	37.75±1.71	31.25±2.22	31.00±2.59	31.00±1.41	30-34
	Post	31.50±1.30	27.75±4.58	32.25±1.71	32.00±1.63	31.50±1.73	31.25±1.90	
Neutrophils (%)	Pre	41.50±5.07	42.50±5.45	43.00±8.41	46.00±4.09	43.25±6.19	41.25±6.02	28-47
	Post	38.75±3.10	51.00±2.71	42.25±5.32	38.75±2.87	48.00±5.94	42.00±11.52	
Lymphocytes (%)	Pre	55.00±5.35	54.25±5.12	54.25±9.00	51.00±4.24	54.50±5.92	55.25±5.91	39-62
	Post	56.00±2.16	45.75±5.32	54.00±5.72	58.75±4.57	49.25±6.65	53.75±10.15	
Monocytes (%)	Pre	1.50±0.53	0.75±0.96	0.75±0.96	1.00±0.82	1.00±0.82	1.50±1.29	2-10
	Post	2.25±1.26	1.25±0.96	1.75±1.50	1.25±1.89	1.50±0.58	1.00±0.82	
Eosinophils (%)	Pre	0.50±1.00	2.00±1.41	1.25±0.96	1.25±0.50	1.25±0.96	0.25±0.50	0.5-11
	Post	1.50±0.58	1.25±0.50	1.00±1.16	0.25±0.50	0.75±0.50	2.00±0.82	
Basophils (%)	Pre	0.50±1.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.25±0.50	0-2
	Post	0.00±0.00	0.25±0.50	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	

Table 5.2 (Continue).

Item		**control**	**antibiotic**	***T. indica***	***M. indica***	***A. indica***	**acetic acid**	**Reference range**
ALT (U/L)	Pre	39.00±5.94	51.25±4.99	45.50±11.73	37.00±4.90	40.25±9.43	52.00±2.71	31-58
	Post	41.25±9.91	37.00±7.39	46.00±8.71	46.00±5.35	40.25±10.34	40.75±6.19	
AST (U/L)	Pre	43.50±10.47	51.75±15.59	35.25±23.31	54.25±13.94	54.50±11.70	57.25±13.84	32-84
	Post	55.75±9.22	54.75±18.52	51.75±11.84	48.50±7.42	43.00±8.45	64.25±8.54	
Creatinine (mg/dL)	Pre	1.68±0.53	1.50±0.52	1.98±0.40	1.93±0.61	1.98±0.05	1.93±0.36	1.0-2.7
	Post	1.53±0.69	1.85±0.52	1.98±0.63	1.73±0.30	1.53±0.38	1.60±0.81	

Values are shown as mean ± SD.

^{a,b} = Values in each row with different superscript were significantly differ. (P<0.05)

^{x,y} = Values in each column with different superscript were significantly differ. (P<0.05)

Table 5.3 Fecal bacterial population of weaned piglets in different treatment groups (Log10 cfu/mL).

Item	control	antibiotic	*T. indica*	*M. indica*	*A. indica*	acetic acid	*P*-value
Total *Lactobacillus*							
Day 1	2.15±0.16	2.05±0.15	2.08±0.17	2.07±0.13	2.14±0.08	2.03±0.17	0.60
Day 7	2.12±0.15	2.03±0.13	2.02±0.07	2.05±0.19	2.11±0.07	2.12±0.19	0.67
Day 14	2.15±0.09	2.00±0.08	2.03±0.08	1.99±0.15	2.04±0.13	2.04±0.15	0.24
Day 21	2.16±0.04	2.01±0.10	2.08±0.15	2.09±0.06	2.12±0.05	2.08±0.08	0.10
Day 28	2.16±0.02^{a}	1.93±0.09^{c}	2.03±0.06^{b}	2.06±0.05^{b}	2.18±0.04^{a}	2.08±0.06^{b}	0.00
Total *Escherichia coli*							
Day 1	1.87±0.19	1.89±0.21	1.89±0.11	1.90±0.13	1.88±0.24	1.92±0.15	0.10
Day 7	1.95±014	1.86±0.12	1.89±0.12	1.90±0.08	1.89±0.09	1.84±0.14	0.69
Day 14	1.97±0.17	1.91±0.17	1.90±0.13	1.91±0.14	1.94±0.14	1.96±0.14	0.95
Day 21	2.00±0.15	1.95±0.14	1.97±0.12	1.98±0.13	1.99±0.16	1.97±0.16	0.10
Day 28	2.06±0.08	1.86±0.11	2.03±0.13	1.97±0.11	2.04±0.14	2.02±0.13	0.06

Table 5.3 (Continue).

Item	control	antibiotic	*T. indica*	*M. indica*	*A. indica*	acetic acid	*P*-value
Total *Lactobacillus*							
Day 1	2.15±0.07	2.05±0.08	2.05±0.17	2.05±0.07	2.08±0.09	2.04±0.06	0.43
Day 7	2.05±0.05	2.04±0.07	2.09±0.05	2.09±0.05	2.03±0.11	2.09±0.09	0.43
Day 21	2.03±0.06	2.04±0.05	2.00±0.11	2.02±0.08	2.08±0.08	2.06±0.09	0.60
Day 28	2.01±0.06	2.06±0.09	2.04±0.02	2.03±0.03	2.03±0.08	2.05±0.05	0.71

Values are shown as mean ± SD

[a,b] = Values in each row with different superscript were significantly differ. (P<0.05)

show a statistical significant difference. The average total *Lactobacillus* species was $\log_{10}$ 2.06, 2.05, 2.05, 2.05, 2.06 and 2.06 CFU/mL, respectively. No statistically significant difference was found on total *Lactobacillus* species (P>0.05).

5.4.4 Gross anatomical observations

The weight of the heart were lighter in *T. indica* wood vinegar, *A. indica* wood vinegar and acetic acid than the control group. No specific differences were found in the weights of the other internal organs, with all the experimental groups showing results similar to the control group (Table 5.4).

5.4.5 Light microscopy observations

Inspection by LM of the intestinal villus morphology (Table 5.5) showed alterations in the villus height. For the duodenum, the villus heights in antibiotic, *T. indica* wood vinegar, *M. indica* wood vinegar, and acetic acid group were significantly higher than control group (P<0.05). The duodenal villus height of *A. indica* wood vinegar was not significantly different than control group (P>0.05). For the jejunum and ileum, the villus heights in antibiotic, *T. indica* wood vinegar, *M. indica* wood vinegar, *A. indica* and acetic acid group were significantly higher than control group (Figure 5.1a). The duodenal, jejunal and ileal crypth depth of piglets did not differ between group (Figure 5.1b). However, the villus height and crypt depth ratio (VH:CD) in duodenum, jejunum and ileum in treatment group was significantly increased (P>0.05). The villus height and crypt depth ratio (VH:CD) in duodenum of *A. indica* wood vinegar was not different than control group (Figure 5.1c). Figure 5.2-Figure 5.3 demonstrates the duodenal villus height and crypt depth measurement of weaned piglets in different treatment groups.

Table 5.4 Weight of internal organ of weaned piglets in different treatment groups.

Item	control	antibiotic	*T. indica*	*M. indica*	*A. indica*	acetic acid	*P*-value
Internal organ (g)							
Liver	764.92±211.86	694.98±98.94	616.68±114.47	724.65±10.71	602.28±99.89	721.52±63.20	0.34
Rt-kidney	70.09±22.19	63.68±14.44	60.45±7.07	72.34±11.07	66.77±12.06	59.05±4.81	0.67
L-kidney	69.89±20.94	62.98±13.06	57.62±5.63	72.07±7.89	65.18±14.24	59.49±8.72	0.57
Stomach	415.24±202.36	353.12±23.79	550.80±255.47	427.31±45.46	341.47±128.92	497.30±43.71	0.33
Duodenum	55.38±10.85	52.52±3.75	51.36±5.53	53.84±10.60	53.74±11.17	57.77±3.47	0.91
Jejunum	1,494.25±509.47	1,427.03±272.71	1,059.18±363.22	1,338.40±138.57	1,255.74±238.36	1,172.04±256.62	0.43
Ileum	23.49±10.05	18.30±4.51	19.21±11.51	20.11±6.56	19.53±5.60	18.08±5.68	0.93
Large intestine	946.12±187.16	1,061.07±65.03	823.44±241.66	675.67±336.39	782.31±186.50	963.78±228.99	0.21
Heart	154.50±37.85^{a}	124.77±25.10^{ab}	106.94±10.55^{b}	126.85±10.91^{ab}	108.03±12.20^{b}	109.19±9.76^{b}	0.03
Spleen	55.46±10.36	52.17±11.17	40.64±6.93	50.04±9.54	42.74±17.26	50.90±10.68	0.44
Lung	258.71±35.08	245.12±24.09	245.94±61.51	295.52±49.04	235.44±55.88	281.87±51.75	0.47

Values are shown as mean ± SD

^{a,b} = Values in each row with different superscript were significantly differ. (P<0.05)

Table 5.5 Intestinal morphology of weaned piglets in different treatment groups.

Item	**control**	**antibiotic**	***T. indica***	***M. indica***	***A. indica***	**acetic acid**	***P*-value**
Villus height (µm)							
Duodenum	461.46±2.00^{c}	510.50±6.40^{b}	498.01±3.64^{b}	493.57±2.31^{b}	474.37±5.65^{c}	529.68±9.74^{a}	0.00
Jejunum	463.16±2.75^{b}	508.34±9.80^{a}	506.92±1.40^{a}	503.86±5.97^{a}	502.40±1.92^{a}	516.58±7.28^{a}	0.00
Ileum	301.43±1.03^{c}	401.68±2.24^{a}	397.77±1.96^{a}	396.35±1.28^{a}	364.98±2.43^{b}	401.00±3.92^{a}	0.00
Crypth depth(µm)							
Duodenum	124.18±1.12	124.68±2.01	121.43±1.87	123.79±0.30	126.02±0.80	122.11±1.80	0.29
Jejunum	121.96±1.75	119.96±1.90	121.26±1.65	121.88±1.72	121.76±1.88	122.14±1.90	0.96
Ileum	126.02±0.80	123.26±1.58	121.50±1.62	123.18±1.28	123.51±0.92	123.41±1.31	0.32
Villus height :							
Crypth depth							
Duodenum	3.72±0.02^{c}	4.10±0.06^{b}	4.10±0.05^{b}	3.99±0.03^{b}	3.77±0.06^{c}	4.34±0.14^{a}	0.00
Jejunum	3.80±0.07^{b}	4.24±0.11^{a}	4.18±0.06^{a}	4.14±0.06^{a}	4.13±0.07^{a}	4.23±0.01^{a}	0.00
Ileum	2.39±0.02^{c}	3.26±0.04^{a}	3.28±0.05^{a}	3.22±0.04^{a}	2.95±0.16^{b}	3.25±0.06^{a}	0.00

Values are shown as mean ± SE

^{a,b,c} = Values in each row with different superscript were significantly differ. (P<0.05)

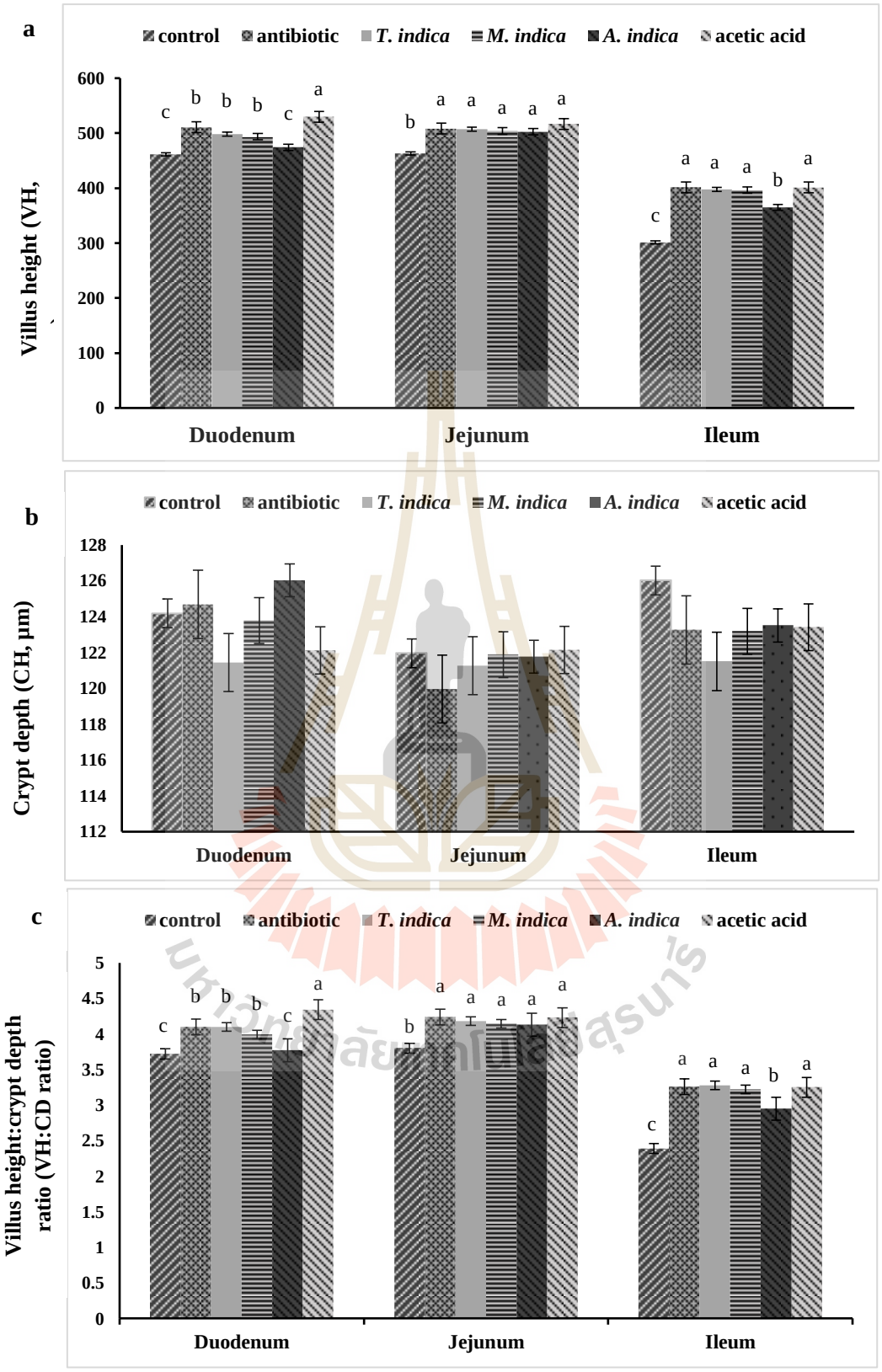

Figure 5.1 (a) Intestinal villus height; (b) crypt depth; and (c) villus height and crypt depth ratio in duodenum, jejunum and ileum of piglets in different treatment groups.

5.4.6 Scanning electron microscopy observations

On the duodenal villi tip surfaces of the control (Figure 5.4a) was covered with comparatively protuberated epithelial cells and microvilli, resulting in an inflated rough surface. Cell outlines were found between the different each epithelial cells. On the villus tip of the antibiotic group (Figure 5.4b), clear cell protuberances developed, covering the villus apical surface and resulting in an inflated rough surface. However, in *T. indica* wood vinegar (Figure 5.4c), *M. indica* wood vinegar (Figure 5.4d) and acetic acid groups (Figure 5.4f), cell protuberances developed, covering the villus apical surface and resulting in an inflated rough surface. In particular, the dome shaped microvilli cells of the antibiotic group, *T. indica* wood vinegar, *M. indica* wood vinegar and acetic acid groups protruded further into the lumen compared with control, a clear cell outline appeared in these treatments. In the case of the *A. indica* wood vinegar group (Figure 5.4e), epithelial cells developed some protuberances, clear outlines, resulting in a smooth villus apical surface. On the jejunal villi tip surfaces, piglets from treatment group (Figure 5.5b-f) showed higher protuberated epithelial cells and microvilli than piglets from control group (Figure 5.5a). These results suggested that all treatment should have had effect on intestinal mucosa, increase in absorption area, since higher intestinal mucosa region was exposed to intestinal lumen.

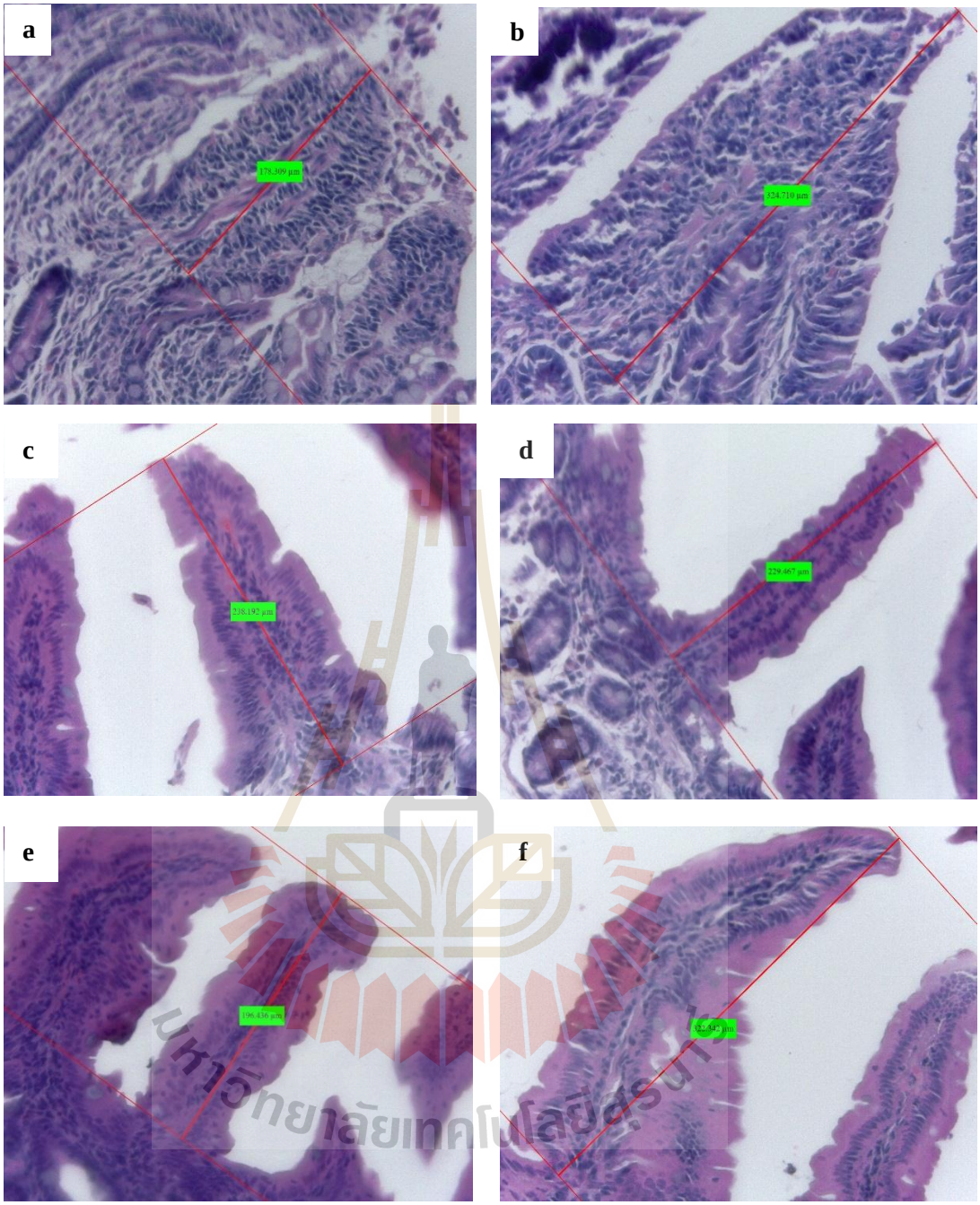

Figure 5.2 Photographic of duodenal villus height of piglets in different treatment groups: (a) control; (b) antibiotic; (c) *T. indica* wood vinegar; (d) *M. indica* wood vinegar; (e) *A. indica* wood vinegar and (f) acetic acid

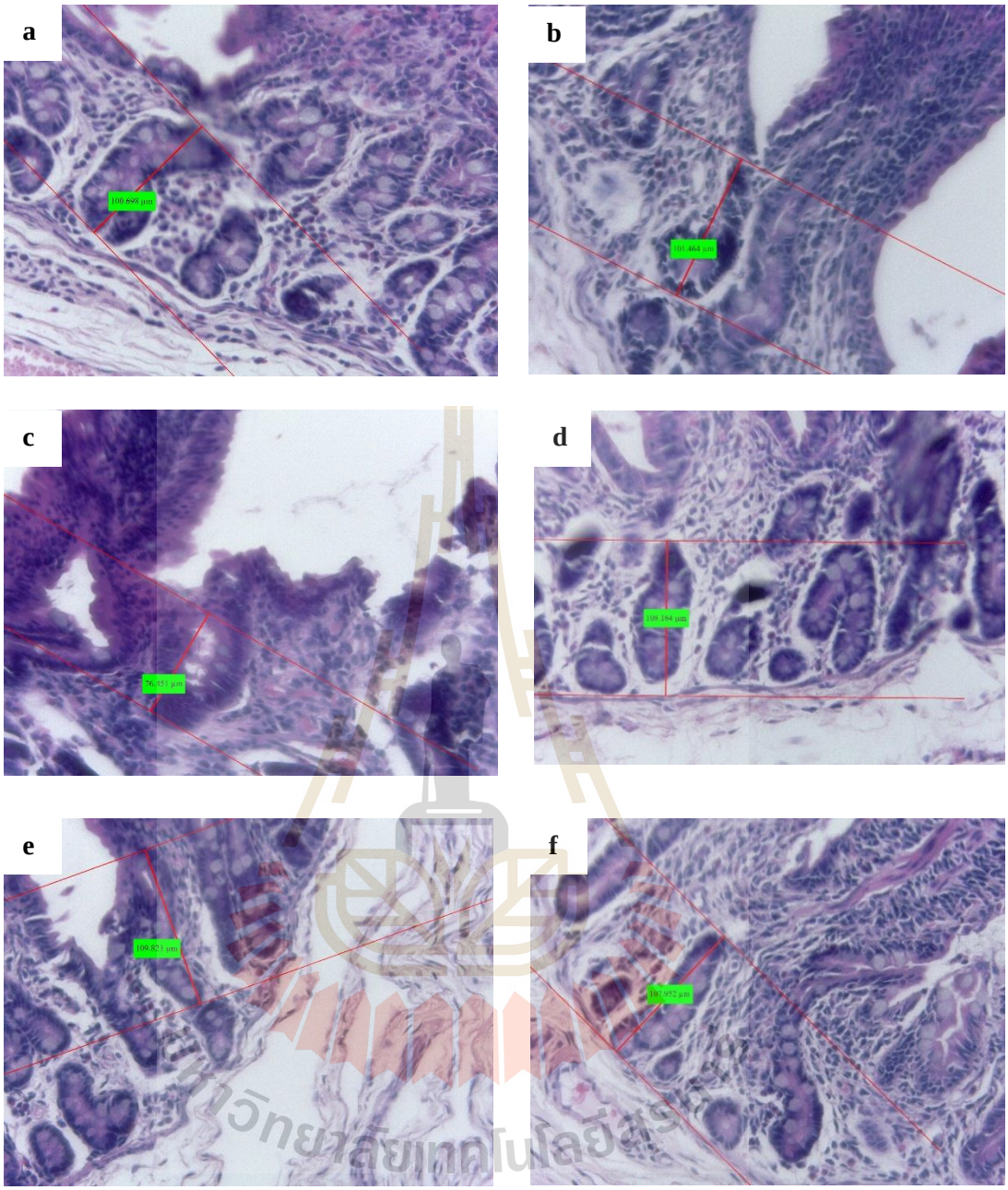

Figure 5.3 Photographic of duodenal crypt depth of piglets in different treatment groups: (a) control; (b) antibiotic; (c) *T. indica* wood vinegar; (d) *M. indica* wood vinegar; (e) *A. indica* wood vinegar and (f) acetic acid.

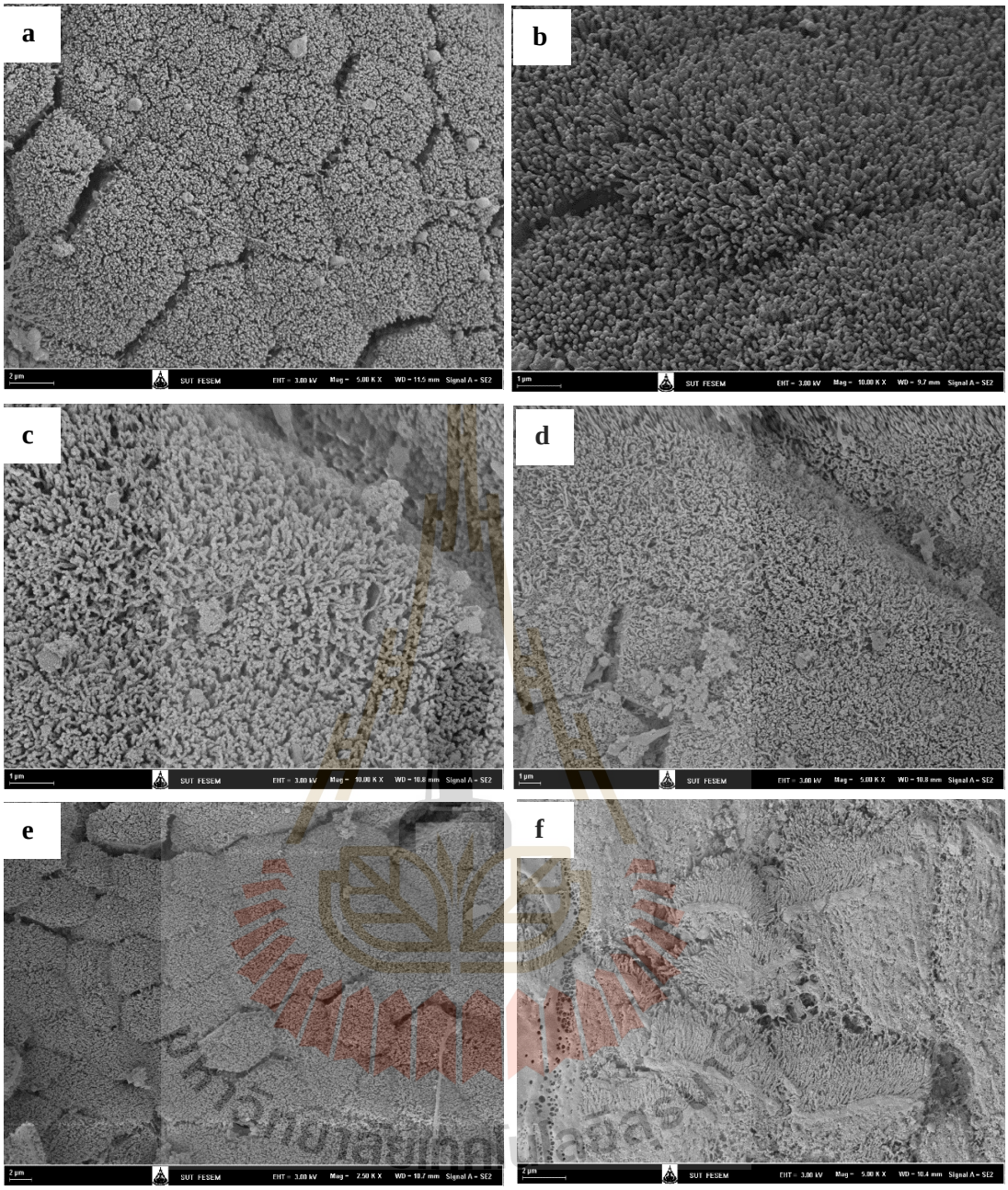

Figure 5.4 Duodenal villi tip surfaces of piglets in different treatment groups: (a) control; (b) antibiotic; (c) *T. indica* wood vinegar; (d) *M. indica* wood vinegar; (e) *A. indica* wood vinegar and (f) acetic acid.

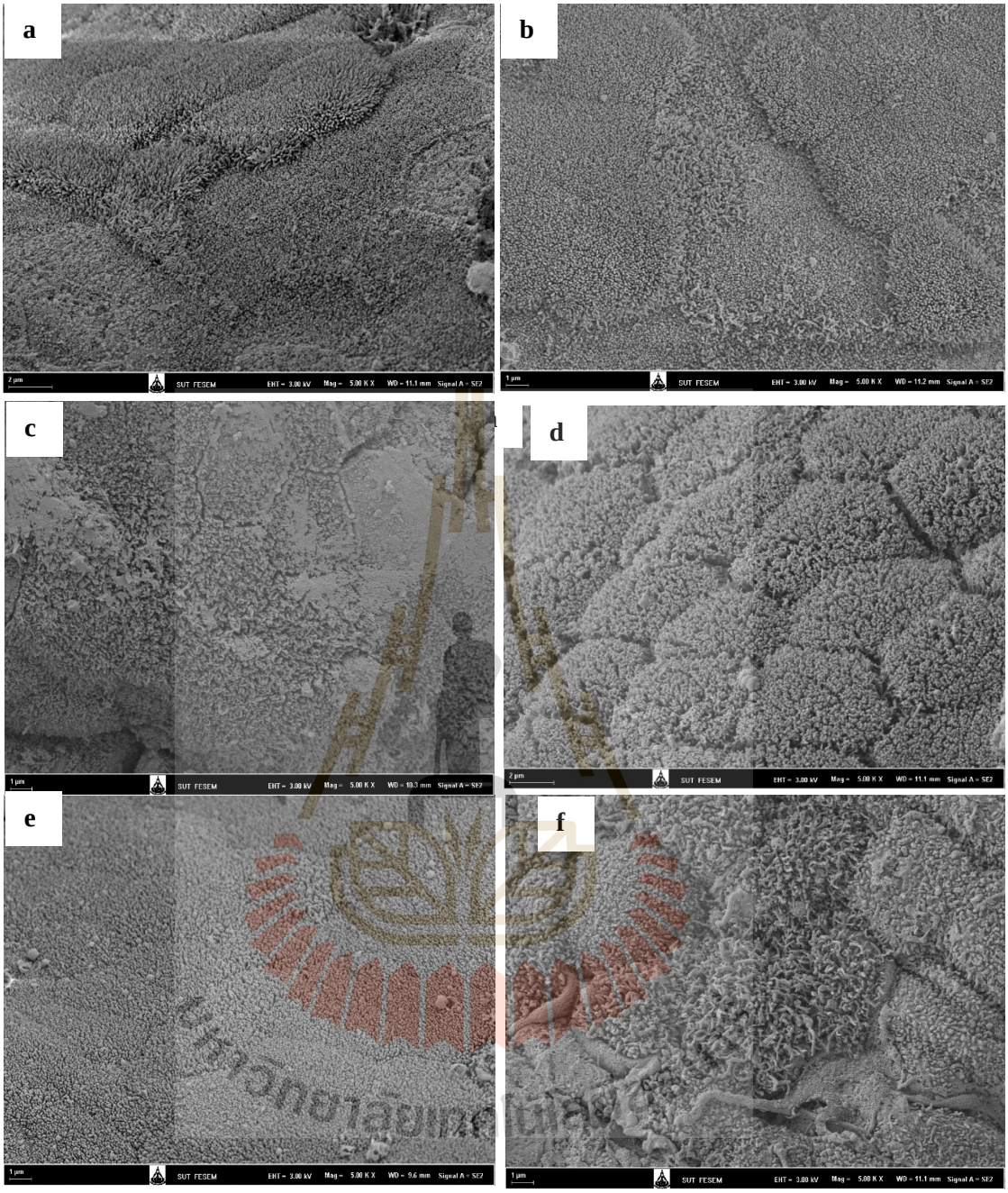

Figure 5.5 Jejunal villi tip surfaces of piglets in different treatment groups: (a) control; (b) antibiotic; (c) *T. indica* wood vinegar; (d) *M. indica* wood vinegar; (e) *A. indica* wood vinegar and (f) acetic acid.

5.5 discussions

5.5.1 Growth performance

There has been growing concern about the negative effect of the use of antibiotics in pig diets (Monroe and Polk, 2000). Therefore, numerous studies are being conducted to find alternatives to replace antibiotics in diets for young pigs. It has been reported that wood vinegar also shows strong acid activity at pH 3 and contains 280 different components, the major ones being acetic and propionic acid (Kim, 1996) and antioxidant substances like phenolic compounds (Loo et al., 2008) thus wood vinegar is also termed as natural organic acids (Sasaki et al., 1999).

In this study, there were no significant differences in body weight gain between the control group and treatment groups. The piglets treated with mango wood vinegar had shown final body weight, feed intake and average daily feed intake significantly higher than other group. However, feed efficiency in treatment groups (antibiotic, *A. indica* wood vinegar and acetic acid) decrease than control group. The piglets fed diets with added oral growth promoters in the form of antibiotics, acetic acid and *A. indica* wood vinegar had better feed efficiency than pigs fed diets devoid of growth promoters. Nevertheless, feeding of acetic acid and antibiotics has resulted in the best growth performance of pigs. Improvements in the performance of pigs fed with antibiotics have been well documented (Doyle, 2001; Li et al., 2008). Several studies reported previously that dietary acidification might be beneficial for the performance of pigs because organic acids consist of one proton and one anion. The effect of the proton of an acid is acidification of the feed while the anion inhibits the growth of microbes (Schutt, 2011). Another important effect is a low gastric pH that optimizes the pepsin activity and improves the digestibility of protein and decreases

the rate of gastric emptying. Organic acids that stimulate exocrine pancreatic secretion of enzymes and bicarbonate improve protein and fat digestion (Luckstadt and Mellor, 2011). Furthermore, organic acids enhance the apparent total tract digestibility and improve growth performance in weaned pigs and improve the production indices (Suiryanrayna and Ramana, 2015). However, there were also studies showing that wood vinegar had positive achievements of supplementation in animal feed. Choi et al. (2009) reported that different levels of wood vinegar added to the diets as dietary treatments (0, 0.1, 0.2, and 0.3%) found that apparent fecal digestibility of dry matter, gross energy, and crude protein was significantly higher (P<0.05) in pigs fed the control diet and the performance of pigs fed wood vinegar was superior to those fed organic acid. Moreover, Yan et al. (2012) found that the use 0.1 and 0.2% of bamboo vinegar led to a greater apparent total tract digestibility of dry matter and nitrogen (P <0.05) than pigs fed a control diet. It is concluded that the wood vinegar does not directly affect stimulation on growth performance in weaned piglets. Therefore, the effect of wood vinegar on production efficiency depends on other factors such as type and level of wood vinegar, period of experiment time, health status and farm sanitation systems.

5.5.2 Fecal Bacterial Population

In this study, the supplementation of *T. indica* and *M. indica* wood vinegar reduced the fecal total bacteria concentration on day 28 relative to the control. No statistically significant difference was found on total *E. coli* and *Lactobacillus* species. The antimicrobial effect of bamboo vinegar in reducing fecal coliforms was reported by Chu et al. (2013) and Yan et al. (2012) as an alternative to antibiotics to reduce fecal pathogens in pig diets. Choi et al. (2011) reported that antibiotics and probiotics could improve nutrient digestibility by allowing more efficient intestinal

growth and improving intestinal microbial balance. The possible cause of improved nutrient digestibility in which could reduce the intestinal pH and the concentration of *E. coli*, and thereby might increase secretion of gastric enzyme and reduce the microbial competition with the host for nutrient.

5.5.3 Light microscopy and scanning electron microscopic alterations

Intestinal morphology is an important indicator for probiotic supplementation. The intestine is the site of nutrient absorption. Better villus height presumes an improvement in the absorptive ability of small intestine, as it has been suggested that the prevalence of long villi results in an increased surface area capable of greater absorption of available nutrients (Caspary, 1992). In fact, intestinal morphology is markedly affected by the diets fed to animals (Langhout *et al*., 1999). In this study, long villi were observed in the treatment groups. The wood vinegars enhance villus height and VH:CD ratio in duodenum, jejunum and ileum except in *A. indica* wood vinegar was not different than control group. An increased villus size provides greater absorptive surface area and a better capacity for absorbing available nutrients. Gilmore and Ferretti (2003) reported that villus height is increased by the enhanced efficiency of digestion and absorption in the small intestine, when a population of beneficial bacteria supplies nutrients and stimulates vascularisation and development of the intestinal villi. Intestinal crypts are invaginations of the epithelium around the villi, and are lined by epithelial cells which secrete enzymes. The base of the crypts is constantly dividing to maintain the structure of the villi. Therefore, an increase in crypt depth would produce more developed villi. However, this experiment showed a no significant increase of crypt depth of piglets treated with wood vinegar compared to the control group. Therefore, this kind of morphological changes induced in the intestine can indicate the effect of growth promoters on changes in the level of

intestinal absorption and hence the change in the performance of piglets. The results obtained from this study showed supplementation of wood vinegar significantly improved intestinal growth and development, although the results of growth performance did not differences than control group.

The duodenum and jejunum are an important organ that absorbs nutrients and is easily destroyed by other microorganisms and chyme as was observed by SEM. In the SEM observations, very large protuberated epithelial cells and microvilli were found on the duodenal and jejunal tip surface in the antibiotic, *T. indica* wood vinegar, *M. indica* wood vinegar and acetic acid groups. In addition, protuberated cells were also reported in piglets showing high nutrient digestibility (Mekbungwan et al., 2004). However, such morphological features become faint in treatment groups. The result for the treatment group is not clear and might have been a result of a poor sampling protocol.

5.6 Conclusion

The current study revealed that piglets treated with wood vinegar and acid supplementation for improves growth performance during 21-48 days of age. No effects of wood vinegar and acid supplementation on growth performance. The piglets treated with *T. indica* and *M. indica* wood vinegar had reduced the fecal total bacteria concentration on day 28 relative to the control. The wood vinegars improved intestinal morphology by enhance villus height and VH:CD ratio in duodenum, jejunum and ileum except in *A. indica* wood vinegar was not different than control group. In the SEM observations, very large protuberated epithelial cells and microvilli were found on the duodenal and jejunal tip surface in the same treatment groups. Another

important finding of the present study was the reduction in heart weight by treatment with wood vinegar. The hematological traits and chemical profile of piglets fed the experimental treatments are normal ranges.

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CHAPTER VI

OVERALL CONCLUSIONS AND IMPLICATIONS

6.1 Conclusions

This study aimed to investigate whether feeding of *Mangifera indica*, *Tamarindus indica* and *Azadirachta indica* wood vinegars to piglets can inhibition the growth of pathogenic bacteria and improve their performance. For these purpose, the study was carried out comprising 3 experiments. The first experiment was conducted to determines the chemical composition of wood vinegar from *M. indica*, *T. indica* and *A. indica*, using GC-MS (Chapter 3). The next experiment was done to evaluate the antibacterial activities (*Staphylococcus aureus* TISTR 746*, Escherichia coli* TISTR 073*, Staphylococcus aureus* ATCC29213, Methicillin-resistant *Staphylococcus aureus* (MRSA) and *Escherichia coli* ATCC25922) using agar disc diffusion and broth microdilution methods, including the mode of action of each wood vinegars on *Escherichia coli* TISTR 073 was determined by SEM (Chapter 4). The last experiment was conducted to evaluate as an alternative to on growth performance, fecal bacterial communities, intestinal morphology and blood characteristics in weaned piglets (Chapter 6).

In conclusion, All wood vinegars were collected at the exit of a vapor funnel during the process of carbonization, with temperature range between 270°C to 393°C within 90 to 120 minutes. The wood vinegars separated from the middle layer had smoke odor and transparent black to brown color and had distillation temperature at

70°C. The pH values of wood vinegars were 3-4. The results reveal that wood vinegars showed the presence of key bioactive compounds, especially acetic acids with a range of 18.6 to 29.45%. The others composition of wood vinegar were phenols, ketones, ester, benzene and its derivatives, aldehydes, alcohols, and some sugar derivatives. The results of antibacterial activity of the vinegar against Gram-positive and Gram-negative pathogenic bacteria was exhibited broad spectrum inhibition. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration values were similar with ranging from 1.25 to 6.25 %v/v. The electron micrographs of cells treated with each wood vinegars showed the similar activity by the disrupted cell wall and membranes in Gram-negative bacteria. Microstructural observations showed that wood vinegars appeared depletion of the content of bacterial cells, indicating that the cell structures of treated bacteria were severely affected and damaged by the antibacterial agent. The treated cells showed an incomplete and deformed shape of the cells when compared with the untreated cells. The piglets treated with *T. indica* and *M. indica* wood vinegar at MIC 6.25 orally 0.5-1 ml/20 kg bodyweight 28 days had reduced the fecal total bacteria concentration on day 28 relative to the control. No effects of wood vinegar and acid supplementation on growth performance. The wood vinegars improved intestinal morphology by enhance villus height and observations very large protuberated epithelial cells and microvilli on the duodenal and jejunal tip surface. The hematological traits and chemical profile of piglets fed the experimental treatments are normal ranges. All experimental results were carried out on standard farms, which tested under other conditions, such as diarrhea pigs may be more obvious.

6.2 Implication

In the recent years, incidence of multidrug resistance in pathogenic and opportunistic bacteria has been increasingly, there researcher has been increasing concern that the use of antibiotics in food producing animals, particularly their long term use for growth promotion, contributes to the emergence of antibiotic resistant bacteria in animals. The results of our study showed antibacterial activity against clinical antibiotic-resistant pathogens, which implies they can be developed into useful sterile products for medical, aquaculture, and livestock breeding applications. The results suggest the antibacterial activities of *T. indica, M. indica* and *A. indica* wood vinegars are contributed by major component of wood vinegar products are acids such as acetic, propioinic, butanoic acid and phenol compounds in various concentrations. The chemical constituents of the wood vinegar must be identified to maximize utilization. *T. indica* and *M. indica* wood vinegars treated to weaned pigs improved the intestinal morphology. In particular, its enhance villus height and observations very large protuberated epithelial cells and microvilli on the duodenal and jejunal tip surface. Therefore, the results suggest that *T. indica* and *M. indica* wood vinegar possible be used as an alternative potential additive to increase the efficiency of intestinal absorption and hence the change in the performance of weaned piglets. However, The period of growth in experiments, treated with diarrhea piglets were important for study the properties of wood vinegars.

BIOGRAPHY

Ms. Pimchanok Lohtongkam was born on 11th January 1981 in Lopburi, Thailand. In 2004, she obtained her Bachelor's degree in Docter of Veterinary Medicine from the Faculty of Veterinary Medicine, Kasetsart University, Bangkok. She has been working as a lecturer at the Department of Veterinary Technology, Faculty of Science and Technology, Nakhon Ratchasima Rajabhat University, Nakhon Ratchasima since 2009. In 2012, she was awarded got a scholarship by the Nakhon Ratchasima Rajabhat University for her Doctor of Philosophy (Ph.D. degree) study in Animal Production Technology at the School of Animal Technology and Innovation, Institute of Agriculture Technology, Suranaree University of Technology with the thesis entitled "Study of Thai wood vinegar as antibiotic alternative on diarrhea prevention and growth performance in weaned piglets". The party results of her Ph.D. thesis have been present in The 67th International Congress and Annual Meeting of the Society for Medicinal Plant and Natural Product Research (GA 2019) on September 1-5, 2019 at Innsbruck, Austria. Austria. p247.