SYNTHESIS AND CHARACTERIZATION OF LACTIC ACID GRAFTED *EUPHORBIA TRIGONA* MILL ANTI-BACTERIA ADHESIVE

Yemisrach Gezahegn Shibeshi

A Thesis Submitted to

The Department of Chemical Engineering

School of Mechanical, Chemical and Materials Engineering

Presented in Partial Fulfillment of the Requirement for the Degree of Master's in Chemical Engineering (Specialization in Process Engineering)

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Adama, Ethiopia

Synthesis and Characterization of Lactic Acid Grafted *Euphorbia trigona* Mill Latex Anti-Bacterial Adhesive

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Office of Graduate Studies

Adama Science and Technology University

October, 2021

Adama, Ethiopia

DECLARATION

I hereby declare that this Master Thesis entitled “Synthesis and Characterization of Lactic Acid Grafted *Euphorbia trigona* Mill Latex Anti-Bacterial Adhesive” is my original work. That is, it has not been submitted for the award of any academic degree, diploma, or certificate in any other university. All sources of materials that are used for this thesis have been duly acknowledged through citation.

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We, the advisor(s) of this thesis, hereby certify that we have read the revised version of the thesis entitled “Synthesis and Characterization of Lactic Acid Grafted *Euphorbia trigona* Mill Latex Anti-Bacterial Adhesive” prepared under my/our guidance by Yemisrach Gezahegn Shibeshi submitted in partial fulfillment of the requirements for the degree of Mater’s of Science in Chemical Engineering (Specialization Process Engineering). Therefore, I/we recommend the submission of the revised version of the thesis to the department following the applicable procedures.

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LIST OF ABBREVATIONS

ASTM	American Society for Testing and Materials
AV	Acid Value
BSA	Bovine Serum Albumin
CFU	Colony Forming Concentration
^{13}C	Carbon Nuclear Magnetic Resonance
DSC	Differential Scanning Calorimetry
DTG	First Derivative of TGA
E.coli	Escherichia coli
FT IR	Fourier Transform Infrared Spectroscopy
^{1}H	Hydrogen Nuclear Magnetic Resonance
MHA	Muller Hilton Agar Medium
NH	National Institute of Health
NMR	Nuclear Magnetic Resonance Spectroscopy
RF	Retention Factor
S.aureus	Staphylococcus aureus
TLC	Thin Layer Chromatography
TGA	Thermogravimetric Analysis
TRM	Total Resinous Matter
TSC	Total solid content

LIST OF NOTATIONS

Cp	Centipoise
C	Concentration
ETM	*Euphorbia trigona* Mill latex
ETM-*l*-OLLA 1	1:1 ratio of lactic acid *Euphorbia trigona* Mill latex
ETM-*l*-OLLA 2	1:2 ratio of lactic acid *Euphorbia trigona* Mill latex
ETM-*l*-OLLA 3	1:3 ratio of lactic acid *Euphorbia trigona* Mill latex
m	Mass
mg	Milligram
mM	Mill molar
mL	Milliliter
V	Volume
W	Weight
%	Percent

ABSTRACT

The increased modernization resulted in the need for the simplified living style of human beings which includes the use of smarter materials in day-to-day life. Those materials are mainly plastics which may have adverse effects on the environment, and thus finding greener materials is needed. So, this study aimed to synthesize lactic acid grafted Euphorbia trigona Mill latex anti-bacterial adhesive and characterize the synthesized bio-adhesive in terms of the antibacterial activity, thermogravimetric analysis, and adhesion property. Euphorbia trigona Mill latex was modified with L-Lactic acid (88%) with a mix ratio of 1:3 (V: V) of lactic acid to ETM latex at a reaction temperature of 150ºC. The lactic acid monomer was developed on the backbone of ETM latex through the OH active site. This was confirmed by the FT IR and NMR analysis. The thermal stability of the synthesized bio-adhesive was investigated and compared to the starting materials and found to be more stable with a maximum degradation temperature of 370℃. The in vitro antibacterial test with agar disk diffusion assay was used to investigate the antibacterial activity of the bio-adhesive and it was found that the bio-adhesive has concentration-dependent activity towards E.coli and S.aureus bacteria. For 1wt%, 2wt% and 3wt% of bio-adhesive an increased ant-bacterial activity was observed with a zone of inhibition of 9mm, 13mm, 15mm for E.coli and 8mm, 11mm, 14mm for that of S.aureus respectively. The adhesion nature was investigated by the drag method with an experimental procedure developed in the laboratory. The commercial adhesive bandage was washed with a solvent and used as an adhesive bandage fabric and 0.1gm of ETM-l-OLLA was applied on it and it was glued to a leather substrate. Then the adhesive performance of the ETM-l-OLLA towards peel force was estimated indirectly by applying a known amount of mass. With this experimental procedure the peeling strength for the synthesized bio-adhesive was estimated to be in between of the maximum and minimum weight recorded to detach it from the substrate and it was observed that the adhesive performance increases with the increase of the mix ratio of ETM latex.

Key words: ETM latex, chemical structure, bio-adhesive, anti-bacterial activity, adhesion performance.

CHAPTER ONE

1. INTRODUCTION

1.1.Background of the Study

Humans' day-to-day activity is dependent on the usage of petroleum-derived products like stationery materials, industrial equipment, medical materials, and also for home consumption. Ever since from the first industrial production of synthetic polymers (plastics) around the 1940s' until now their consumption has an increasing trend that is maybe due to their unique property like light-weight, durable, and versatile, allowing their incorporation into a diverse range of applications that bring simplicity to human life. Besides their unique property and advantage of making life simpler, they have the drawback of causing environmental pollution that may arise from their non – degradability, poor disposal after consumption, and their carbon dioxide emission during decomposition (Al-Salem, Lettieri, and Baeyens 2009). So, to suppress this drawback, proper waste recycling is believed to be the well-helping way which in turn be an alternative way of energy recovery. Other than recycling shifting to the more greener and bio-degradable materials termed biomaterials is a current research trend (Ige, Umoru, and Aribo 2012).

Biomaterials are defined by an American National Institute of Health as "any substance or their combination or "completely any tissue part of the body or function of the human body to maintain or improve the individual's quality of life. Metals, ceramics, plastic polymers, or naturally occurring products that are compatible with the human body and the environment are examples of biomaterials(Hossain, Roy, and Guin 2017). They have extensive application in the field of biomedical engineering as joint, dental, and suture replacements, cosmetic corrections and, etc. Biomaterials can be also modified or reengineered, molded, or machined as parts, coatings, films, foams, and fabrics for use in biomedical products and devices (Tathe, Ghodke, and Nikalje 2010). The utilization of biomaterials goes back nearly a *Mill*ennium, and advances have led to smart bio-materials that can be functionalized via pre-polymerization or post-polymerization methods, including functional molecules (Wohlhauser et al. 2018).

The term biomaterial defines both biocompatibility and the source of the material and hence the biomaterials that are produced from nature either plants or animals are known as biopolymers (Ige, Umoru, and Aribo 2012). Plant-based biopolymers can be extracted as essential oils like soya oils

with solvent extraction methods or collected with physical tapping methods like plant latex and undergo appropriate modification to give a value-added product (Hossein et al. 2018).

Plant latex as a source of biopolymer: Latex is a natural plant polymer produced by highly specialized plants. It is a milky substance secreted primarily by lactiferous tissue ducts and flows through roots, stems, leaves, and other parts of laticifiers. Plant latex is known to be the fruit of all blooming plants; which is an emulsion-like sticky substance emitted by certain plant sections following minor tissue damage (Konno 2011). The building blocks or the chemical constituents of almost all latex components are merely reported as C3H3N (carbon, hydrogen, and nitrogen). It contains glycolipids, alkaloids, acids, laticifier proteins, and acid phosphatase from *Euphorbia characias* latex, among other things (Hagel, Yeung, and Facchini 2008).

Terpenoids and their esters stored as globular aggregates enclosed by a thin protein layer are the main constituents in the latex of *Euphorbia* species (Palocci et al. 2003). Terpenoids are oxygenated, hydrogenated, and dehydrogenated plant-derived hydrocarbons with the general formula (C5H8)n. Terpenes are classified into hemiterpenes, hemiterpene's, diterpernes, and sesquisterpenes based on the presence of polyisoprene units in the structure (Krishnaswamy 2010). Monoterpenes like myrcene, pinene, and limonene are examples of directly polymerized terpenes. Other terpenes can be oxidized by Baeyer–Villiger, yielding a lactone that can be polymerized using ring-opening techniques. A variety of processes can be used to polymerize the various terpenes. Monomeric terpene polymerizations can be radical, ionic, or even coordinative (Sahu, Bhowmick, and Kali 2020). Terpenes can undergo chemical transformations such as isomerization, cyclization, hydration, condensation, hydrogenation, dehydrogenation, oxidation, rearrangement, esterification, hydro formylation, and alkylation to produce new polymeric materials. (Schwab, Fuchs, and Huang 2013).

Polymerization: A reaction process in which smaller molecules termed monomers join together to give high molecular weight macromolecule or polymer. Based on of the polymerization mechanism involved to yield the resulting product, polymers can be addition polymers, in which the molecular formula of the structural unit is identical with that of the monomer from which the polymer is formed, and condensation polymers, in which the structural unit lacks certain atoms present in the monomer from which the polymer is formed or to which it can be degraded by chemical means. On the other hand, based on the structure polymers can be branched, linear, or

cross-linked. Those both differences of the formed polymers finally have a generalized effect on their molecular weight which in turn affects the physical and mechanical properties(Flory 1946). So, while synthesizing a polymer or modifying the naturally existing polymer or biopolymer controlling their molecular weight is crucial for their application.

Natural product and their efficacy: Depending on their qualities, biopolymers from various sources such as plants (starch, cellulose, and natural rubber), animals (collagen, hyaluronic acid, chitosan), fungi (chitin), bacteria (bacterial cellulose, exopolysaccharides), and algae (alginate) are utilized. Because of their biocompatibility, biodegradability, and decreased antigenicity, these polymers have an advantage over synthetic materials (Sahana and Rekha 2018). And as the current research world is shifting towards greener materials, finding the non-feed competent and easily available natural product is the other direction that needed to be followed.

1.2.Statement of the Problem

Due to the issue of scarcity, and toxicity towards the environment that arises from the non-degradability of the petroleum-derived materials and poor disposal custom of the consumers, researchers are trying to shift towards greener and environmentally friendless materials that are biomaterials. Biomaterials can be synthetic like metals, ceramics, plastics, or naturally occurring polymers (Ullah and Chen 2020). The naturally occurring biopolymers that are isolated from animals or plants can be modified before being used to synthesize value-added polymer products such as commodities, engineering thermoplastics and fibers, and functional materials in biomedicine. The well-investigated nature-derived polymers include nucleic acids, polysaccharides, proteins, lipids, and complex macromolecules such as proteoglycans that can be derived from non-mammalian and mammalian sources can be used to impart biological activity to a material (Joyce et al. 2021). Other than those widely investigated nature-derived polymer sources plant latex can also be used as a source of bio-polymers as it contains various macromolecules (like terpenes, alkaloids,) that can be isolated via different extraction methods or modified insitu towards value-added products (Agrawal and Konno 2009). From the known modification methods, conjugate development helps to develop derivatives of the base natural polymer under investigation without accommodating the potential bioactivity.

In this study, *Euphorbia Trigona Mill* latex was selected and investigated for the synthesis of bio-adhesive having an anti-bacterial effect. *Euphorbia trigona* Mill plant is available easily and

abundantly in almost all parts of our country including, Oromia, Tigria, Amhara regions. The *Euphorbia trigona* Mill latex can be used as raw material for bio-adhesive as it has a tacky nature with necessary improvements to its properties Moreover, *Euphorbia trigona* Mill plant latex constitutes different secondary metabolites like alkaloids, phenols, flavonoids, tannins, etc. Those listed and other secondary metabolites are known to be potential bioactive components that are used in the production of drugs. This plant has a documented use in traditional medicines in different countries of the world including our country Ethiopia (Enyew et al. 2014). Other than its medicinal value companied with the adhesive nature it's known that the plant is not known for its use as an edible plant this one is the other advantage which encourages to be as a substitute for the synthesis of the bio-based adhesive. *Insitu* polycondensation of L-Lactic acid with *Euphorbia trigona* Mill latex was selected as a modification technique to retain its adhesive nature and elastomeric nature as it coagulates when it is exposed to air.

1.3.Research Question

Research question: The present study aims to answer the following major question;

- ✓ Can *Euphorbia trigona* Mill latex be modified with L-Lactic acid with insitu polycondensation reaction method?
- ✓ Can *Euphorbia trigona* Mill based bio-adhesive have an anti-bacterial effect?
- ✓ What will be the effect of the reaction condition (mix ratio) on the physical property (bulk density and solubility) of the synthesized *Euphorbia trigona* Mill L-Lactic acid bio-adhesive?

Based on the above research question, the research system, methodology, and characterization techniques are proposed. *Euphorbia trigona* Mill latex is selected as the base of study point due to;

- ✓ It is easily available biomass with good secondary metabolite content that can be modified into value-added products.
- ✓ It possesses adhesive properties and has an anti-bacterial effect.

1.4.Objective

1.4.1. General Objective

The general objective of this research work was the Synthesis and Characterization of Lactic Acid Grafted *Euphorbia trigona* Mill Latex Anti-Bacterial Adhesive.

1.4.2. Specific Objectives

The specific objectives were

- ✓ To investigate physicochemical characteristics, preliminary phytochemical screenings and, structure and thermal property of the *Euphorbia trigona* Mill latex,
- ✓ To study, the anti-bacteria activities of the *Euphorbia trigona* Mill latex,
- ✓ To synthesize, lactic acid grafted *Euphorbia trigona* Mill latex bio adhesive,
- ✓ To investigate the interaction of synthesized adhesive towards water and temperature
- ✓ To assess the anti-bacteria characteristics of lactic acid grafted *Euphorbia trigona* Mill,
- ✓ To analyze the adhesion property of lactic acid grafted *Euphorbia trigona* Mill latex bio-adhesive.

1.5.Significance and Benefit of the Study

1.5.1. Significance

In this study, the main work was done to synthesize and characterize *Euphorbia trigona* Mill latex-lactic acid bio-adhesive. The selected feedstock *Euphorbia trigona* Mill latex is non-edible, easily available. *Euphorbia trigona* Mill latex more over seems uninvestigated plant biomass so studying the phytochemical constituent characteristic and chemical structure, its anti-bacterial effect helps further investigation other than bio-adhesive synthesis.

1.5.2. The Benefit of the Study

The main benefits of the study include;

- ✓ Bio-adhesive having an anti-bacterial effect and adhesive nature that can be applied for a medicinal purpose was developed.
- ✓ Ways for research on biomaterial bases as *Euphorbia trigona* Mill latex is an abundant and potential plant possessing polymeric characteristics.

- ✓ Positively affects the community and living standards as it is a substitute for synthetic adhesive it reduces dependency on synthetic material and makes environmentally friendly consumption.

1.6.Scope of the study

The scope of this research work extends to the characterization of the *Euphorbia trigona* Mill latex in terms of physicochemical properties, preliminary phytochemical screening, thermal property investigation, and also chemical structure determination as a feedstock for the synthesis of the elastomeric bio-adhesive. The main focus of this thesis is then development of the elastomeric bio-adhesive and studying anti-bacterial and adhesion property investigation.

CHAPTER TWO

2. REVIEW OF RELATED WORKS

2.1. Introduction to Latex Bearing Plants and Plant latex

2.1.1. Latex Bearing Plants

Higher-level plant families can produce latex that is a sticky aqueous milky-like substance. Plant latex is produced at a plant part known as a laticifier and excluded as a result of tissue damage. Different plant families that produce latex for their purpose may be for a defense mechanism. The chemical composition of latex and the structure of the laticifier is known to be dependent based on plant origin, anatomy, and geographical distribution. It was found that more than 2000 species of around 40 families of angiosperm plants could secrete latex, which constitutes 8.9% of all angiosperm plants (Hua et al. 2017; Konno 2011). From those many plant families, *Euphorbiaceae* is one that contains many species.

Euphorbiaceae, also known as the milkweed family, is a large family of flowering plants with more than 300 genera and more than 7,500 species. It is one of the most complex and diverse families among angiosperms (Hua et al. 2017). Members of the milkweed family exhibit a variety of growth forms, ranging from small, short-lived plants to annual or perennial herbaceous plants, shrubs, small trees, and cactus-like succulents. This plant family includes several plants with good economic value, such as Cassava (*Manihot esculenta*), Paragomero *(Hevea brasiliensis*), Castor bean (*Ricinus communis*), and Barbados nut (*Jatropha curcas*), while some of them such as the Poinsettia (*Euphorbia pulcherrima*) and Spurge (*Euphorbia esula*) are also cultivated as ornamental plants, while others such as *Euphorbia Milii Des Moul, Euphorbia trigona* Mill, and *Euphorbia Antiquorum L* are considered medicinal plants (State 2019).

Euphorbia is the fourth largest genus of flowering plants; it also has one of the largest ranges of chromosome counts, along with Rumex and Senecio. Members of the family and genus are commonly referred to as spurges. The family is primarily found in the tropical and subtropical regions of Africa and the Americas, but also temperate zones worldwide. Succulent species originate mostly from Africa, the Americas, and Madagascar (Abd-Alla et al. 2019; Ali and Boshara 2014; Mahajan and Chaudhari 2014). The plants belonging to this genus are characterized by the milky white latex, which contains lipids, rubbers, resins, sugars, proteins and, enzymes

(Application 2015). They are also considered medicinal plants as the whole plant extract and also latex contains various secondary metabolites and biologically active compounds. On the other, way the latex of different *Euphorbia* species contain skin irritating and carcinogenic compounds (Hammadi et al. 2019). The compounds most relevant to the toxicity and considerable biological activities in *Euphorbias* are diterpenes, especially those with abietane, tigliane, and ingenane skeletons (Bigoniya, Shukla, and Singh 2010).

2.1.2. *Euphorbia trigona* Mill plant (Cathedral Cactus, African Milk Tree, Kulkual)

Euphorbia trigona Mill (kulkual) is a plant that belongs to the *Euphorbia* genus and *Euphorbiaceae* family consisting of 2008 species. Lasting plant, beginning from Central Africa, with more popular names as the African drain tree, cactus, and cathedral cactus-candle. The plant incorporates a straight, thick stem, 3-4 lined, somewhat branched, dark green with lighter green and V-shaped pattern. On the stem peak, the thistles are tall, turned, around 5 mm in length. Among stems and spines are rare, oblong-lanceolate takes off, with the whole margin, which shows up within the spring, but particularly within the best of the stems (Yener et al. 2018).

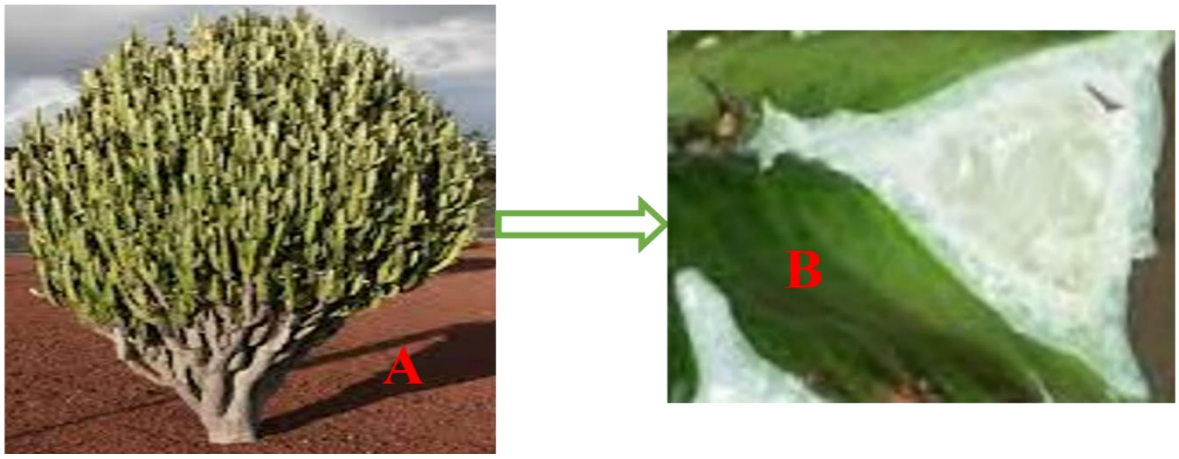


Figure 2. 1: (A) ETM tree (B) ETM tree excluding latex when cut *source: (https://www.google.com/searchLEuphorbia)*

2.2. Plant Latex

Plant latex is a milky be like sap produced by angiosperm plant families in a body part called the laticifier. It is a milky fluid composed of tiny organic droplets suspended in an aqueous medium (a sort of natural colloidal suspension). There are numerous subtypes of latexes based on their

physicochemical properties, but the most well-known are rubbery latexes, which contain polymeric micro-particles that can account for more than half of the latex weight. Because of its sticky nature, it is not used to treat tissue damage caused by herbivorous animals or insects. There has been confusion regarding the exact biological roles of latex were once thought of as a water reserve for the plant's, or the excretion of the plant's waste metabolites. However, scientists are now trying to demonstrate that latex provides defensive roles for plants against insect herbivores and pathogens by gluing the mouthparts or entangling the entire body of herbivores due to its sticky trait (Hua et al. 2017) (Hagel, Yeung, and Facchini 2008). For example, the amount of latex produced by I. brasiliensis and sweet potato (*I. batatas Convolvulaceae*) is determined by light levels, drought, and soil moisture conditions. This was discovered in a recent study of several Asclepias species (Agrawal and Konno 2009).

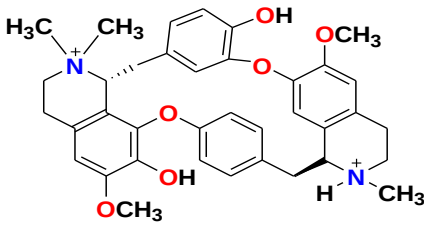
Furthermore, plant latex is characterized by having a diverse range of proteins and specialty products, such as alkaloids, terpenoids, cardenolides, and a variety of other components, the majority of which are toxic to insects and pathogens. The complex factors involved in latex metabolite production, in conjunction with plant biosynthesis, result in chemical differentiation of the latex metabolome from that of other parts of the plant, indicating a high potential as a new bioactive chemical resource (Hua et al. 2017). Now in days, the research world is giving attention to secondary metabolites as they have a diversified role in the field of bio-based material synthesis and also in the field of medicine with accepted medicinal value. Secondary metabolites in plants are classified into three major groups based on their biosynthetic origin: (i) flavonoids and associated to phenolic and polyphenolic compounds, (ii) terpenoids, and (iii) alkaloids and compounds containing nitrogen and sulfur (Crozier, Jaganath, and Clifford 2007).

2.2.1. Major Constituents of Plant Latex

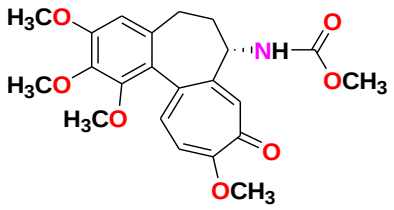
Latex contains an unusually large number of organic compounds, possibly more than any other plant parts, which is a stem, bark, and root extract. Several researchers have studied the lattices from the *Euphorbiaceae* for their biochemical properties such as triterpenes development of plant-based medicines: conservation, efficacy, and safety, polyisoprene, waxes, carboxylic acids, alkanes, and various enzymes (Seshagirirao and Prasad 2001).

Plant proteases: Plant proteases have long been used as anti-helminthic, milk clotting agents, and cheese-making enzymes. Because industrial applications of enzymes necessitate production cost as well as large-scale production, the vast majority of commercial enzymes have been obtained primarily from microbial sources (Shah, Mir, and Paray 2014). Furthermore, plant proteases are more advantageous because they can be used in different pH and temperature conditions. Latex from the Euphorbia genus has been found to contain a variety of proteins and enzymes that can be used in biotechnological applications such as milk clotting (Application 2015), medicinal application [31] [32].

Alkaloids from plant latex: As a constituent of plant latex, alkaloids are irregularly distributed and highly concentrated amongst angiosperm families, including *Apocynaceae*, *Papaveraceae*, and *Moraceae*. Many of the constituents in plant latex have no defined use except that they are thought to act as a plant defense system (Ivanova et al. 2016). Alkaloids demonstrated a wide range of medicinal properties. Many of them have local anesthetic properties, but their real-world application is limited to the clinical level. As a nutrient, they are found in edible plant materials like cacao leaf, coffee, tomato, potato (Salomé Abarca, Klinkhamer, and Choi 2019)



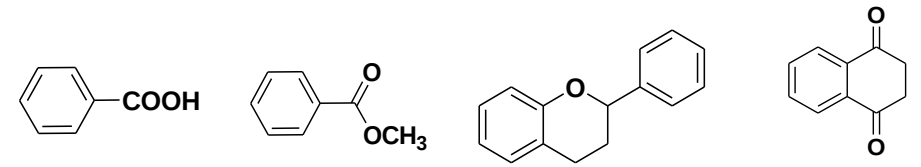
Tubocurarine



Colchicine

Figure 2.2: Some natural alkaloids having medicinal pharmacological use

Phenols and tannins from plant latex: tannins are kinds of stringent, polyphenolic bio-macromolecules that tend to attach to proteins whereas phenols are specifically aromatic compounds that are bonded to a group. Titration methods can be performed for the preliminary detection of both components (Salomé Abarca, Klinkhamer, and Choi 2019). Gallic acid, naphthoic acid, quercetin, chlorogenic acid, and rutin were found in the latex of *Hevea brasiliensis (Euphorbiaceae*).

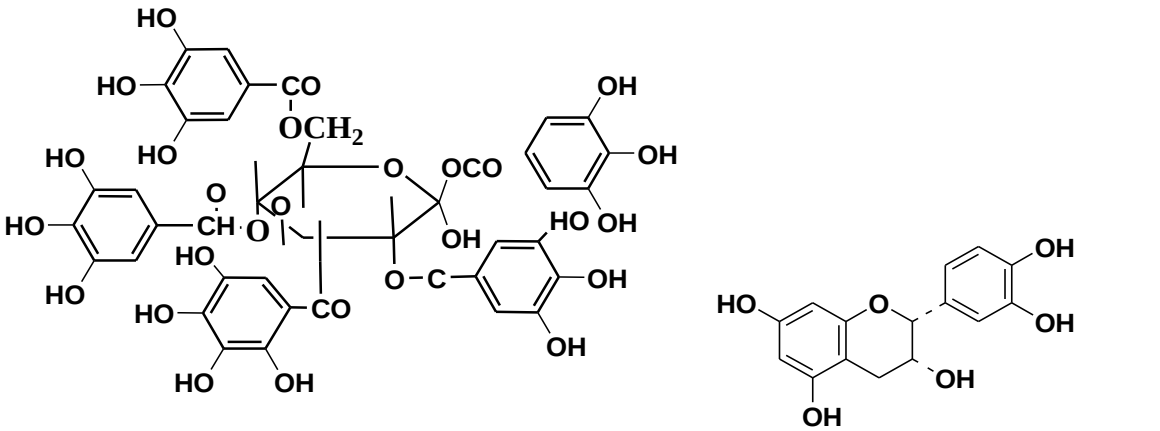


Phenolic acid Acetophenones Flavonoids Naphtoquenone

Figure 2.3: Structural skeletons of some phenolic compounds

Phenolic compounds range in size and sophistication from straightforward, low-molecular-weight, single aromatics to large, complex tannins and polyphenols. They are typically found conjugated to sugars and organic acids and can also be classified based on the number and configuration of their carbon atoms. The flavonoids and non-flavonoids are indeed the two types of phenolic compounds (Crozier, Jaganath, and Clifford 2007).

Tannin is a generic term for a class of polymeric polyphenolic compounds capable of tanning leather or precipitating gelatin from solution. They range in molecular weight from 500 to 3,000 which can be found in nearly every plant part, including bark, wood, leaves, fruits, and also roots. Tannins are categorized into two categories: hydrolyzable tannins and condensed tannins (Koche, Shirsat, and Kawale 2016).



Pentagalloyglose (hydrolyzable tannin) Procyaniidine (condensed tannin)

Figure 2. 4: Structures of tannin skeletal

Hydrolyzable tannins are a combination of phenols such as ellagic acid and gallic acid, esters of sugars like glucose, gallic acid, or digallic acid, and are categorized into two families: gallotannins, which produce gallic acid and its derived products after hydrolysis, and ellagitannins, which produce ellagic acid. Condensed tannins, on the other hand, require 3 to 8 flavonoid repetition units and are composed of associated precursors (flavan-3-ol, flavan-3, 4-diol) of carbohydrates as well as amino and amino acid traces. Condensed tannins typically form complexes with proteins (Keifer and Effenberger 1967).

Saponins from plant latex: Saponins are bitter-tasting compounds that contain both carbon soluble and carbon insoluble carbon atoms. They are compounds with foam-forming properties, and the majority of them are derived from plants. Saponins are hydrolyzed to produce aglycone (sugar) and aglycone (sapogenin). Saponins are categorized as neutral or acid depending on the structural nature of the aglycone or sapogenin. Neutral saponins are derivatives of steroids with spiroketal side chains that are almost exclusively found in monocotyledonous angiosperms, while acid saponins possess triterpenoid structures (Desai, Desai, and Kaur 2009).

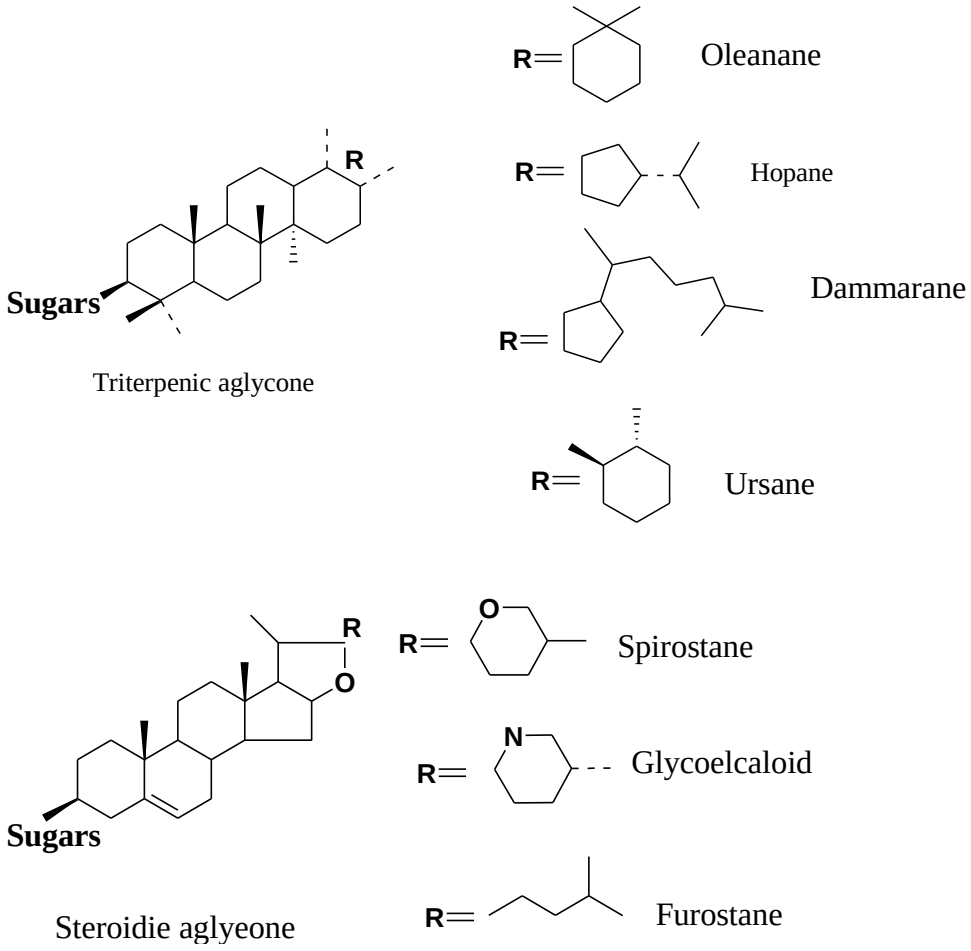
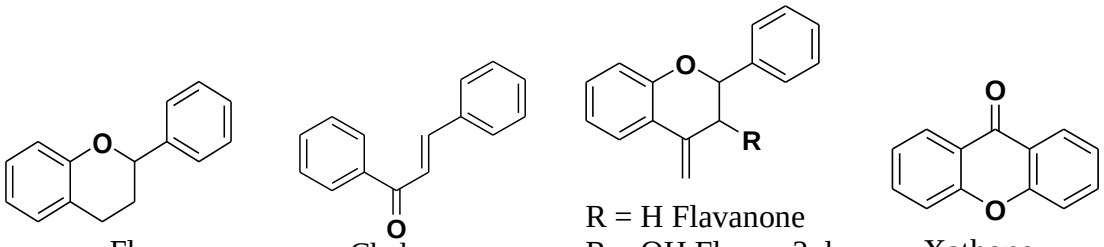


Figure 2. 5: Main possible structure of Steroid agyleone

The skeleton of the steroidic agleone is made up of 27 carbon atoms. These molecules result from an intramolecular acetalization that takes place after oxidation of a cholestanic precursor in C_{16}, C_{22}, and C_{26}, taking into account the spiro-nature of C_{22}; this hex acyclic skeleton is commonly marked by the term spirostane. It is not unusual for hydroxyl in C_{26} to interact with a sugar in fresh plants. The structure could be pentacyclic, which is known as furostane. The triterpenic agleone is produced by cyclizing (3S)-2, 3-epoxy-2, 3-dihydrosqualene. Pentacyclic compounds such as dammaranes, oleananes, ursanes, and hopanes result from this cyclization (Moghimipour and Handali 2015).

Flavonoids from plant latex: are a class bio-organic compound having 15 carbon atom skeletal with the phenyl and heterocyclic rings. Different titration tests including Shinoda's, alkali,

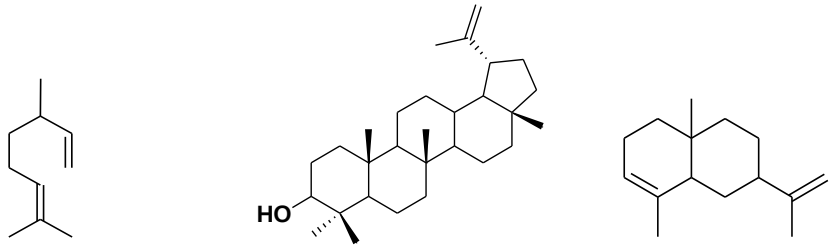
Ammonia, lead acetate test can be used as preliminary tests to detect their presence in a given sample material (Panche, Diwan, and Chandra 2016).



Flavan Chalcones R = H Flavanone R = OH Flavan-3ol Xathone

Figure 2.6: Classes of Flavonoid compounds

Terpenoids from plant latex: terpenoids are a class of organic compounds that are available naturally. They are also called isoprenoids consisting of five carbon atoms or modified terpene in which the group is substituted with an oxygen atom. Terpenes can have different classifications based on the number of isoprene units contained in them. They can be extracted from plants and microorganisms also (Krishnaswamy 2010).



Linalool monoterpene Lupeol pentacyclic triterpene Selinene sesequiterpene

Figure 2.: Some well-known terpenes skeletal

Rubber (Polyisoprene) from plant latex: The term rubber refers to three distinct substances: (a) an elastic solid found in solution in the tissues of a few trees and vines, as Para rubber; (b) an elastic solid found in solution in the tissues of a few shrubs and vines, as Guayule rubber; and (c) a chemical product derived from isoprene or homologous hydrocarbons, as synthetic rubber. Natural rubber (NR) is primarily composed of cis-1, 4-polyisoprene which is generated by over 2000 plant species, the vast majority of which are *Euphorbiaceae* members. Polyisoprene is non-toxic because it is chemically inert. However, when latex coagulates, soluble latex proteins become trapped in poly-isoprene-based latex micelles. This restriction has the potential to be experimental. Natural rubber latex has been made up of 25-30% polyisoprene, 1-1.8% proteins, 1-2%

carbohydrates, 0.4-1.1% neutral lipids, 0.5-0.6% polar lipids, 0.4-0.6 inorganic components, 0.4 percent amino acids, and the remaining 50-70 percent water [40]. But now in a day's people are trying to extract natural rubber from other species of this Euphorbiaceae family as alternative rubber bearing plants.

Table 2. 1: Summary of literature for natural rubber extracted from *Euphorbia* species latex

Species	Extraction method	Characterizations	Result
E.characias(Spanò et al. 2012)	Solvent extraction with cyclo-hexane/ethanol	GPC, FT-IR, NMR	• NR with cis 1, 4-polyisoprene structure. • Optimum rubber yield was with acetic acid as a solvent
E.macroclada (Azadi et al. 2020)	Solvent extraction with hexane/acetone	GPC, FT-IR, NMR	• Low molecular weight NR with cis-1,4 polyisoprene structure
E.caducifolia (I, Khan, and Akhtar 2003)	Soxhlet extraction with acetone	GPC, FT-IR, NMR, DSC	• NR rubber with cis-1,4 polyisoprene structure • Having sticky nature with poor strength • Having a glass temperature of -60.81°C.

2.2.2. Uses of Plant Latex: Medicinal and Bio-Technological Application

Plant latex contains anti-carcinogenic, anti-proliferative, anti-inflammatory, vasodilatory, anti-oxidative, anti-bacteria, anti-parasitic, and insecticidal biologically active chemicals. It is also used in medical sciences to make adhesives, polymers, films, gloves, and other critical diagnostic tools (Agrawal and Konno 2009). The medicinal value of plant latex is attributed to the bio-active chemicals found in plant-like alkaloids, terpenes, and saponins, among other things, which are extracted through a series of isolation and purification methods. Their availability and abundance may differ from plant to plant, as well as from the parts of the plant from which they are extracted, such as the root, bark, leaf, or latex (Ananth et al. 2021). Latex compounds have been proposed for biotechnological applications with relation to rubber substances based on the discovery of the

purification method, structure, and activities. Many latescent species are used in the method of treating a variety of illnesses (Ramos et al. 2020).

The well-known latex-producing plant family *Euphorbiaceae* is known to be a source of bioactive compounds that have both pharmaceutical and biotechnological applications. Proteins from *Euphorbia Trigona Mill* latex were extracted and found to have various medicinal applications like potent antifungal inhibition (van Deenen, Prüfer, and Gronover 2011), ribosome-inactivating (Villanueva, Quirós, and Castañón 2015). The antifungal activity of the latex lectin can be determined by inhibition of fungal conidiospores germination rate and hyphal growth of the filamentous fungi through in vitro analysis. Similarly, (Nashikkar et al. 2011) revealed the anti-swarming mortality, biofilm formation, and virulence factor of *Euphorbia Trigona Mill* latex extracts towards urinary tract infecting bacteria. The effectiveness of the latex towards the test pathogens is due to the bio-active components that act as a quorum sensing compound. Other than those reports, it is also known that this plant latex have a documented use as a traditional medicine for the treatment of different illness in different countries (Gapuz and Besagas 2018; Nashikkar et al. 2011). Even in our country Ethiopia *Euphorbia trigona* Mill is known as a medicinal plant and is being investigated for its bioactive compounds for utilization of drug synthesis based on its medicinal use by local herbalists (Enyew et al. 2014).

The milky latex excluded from higher plant families specifically from Euphorbiacea has also technological application in dairy processing, (Palocci et al. 2003; Smeriglio et al. 2019), agroindustry (Upadhyay 2011) also. Applications towards diary processing involve the extraction of proteolytic enzymes or using the crude protein extract from the plant latex for milk clotting and cheese forming. A proteolytic enzyme applied for milk clotting was extracted from the latex of *Euphorbia trigona* Mill with a simple spectrometry method of 595nm. The extracted proteolytic enzyme activity was characterized for its biochemical activity through the application of milk clotting.

Table 2. 2: Summary of literature review for plant latex uses

Latex Cpds	Plant species	Isolation method	Application
Proteolytic enzymes (Mahajan and Chaudhari 2014)	E.triculli, E.nerifolia, E.nivulia	• The spectrophotometric method at a wavelength of 600nm	• Cheese-making
RIP (Villanueva, Quirós, and Castañón 2015)	E.trigona Mill	• Centrifuged (at 7000g for 1 hour), dialyzed (with PBS buffer) supernatant was fractionated by NH_3 74 sulfate precipitation (40–90% saturation • applied to a Mono Q HR column & eluted in a stepwise 78 gradient using 98, 123, and 250 mM NaCl	• RIPs with cytotoxic activity toward human cancer cell lines.
Protease (trigonin 1, 2 ,3,) (Fitria 2013)	E.trigona Mill	• The crude extract was purified by Gel filtration with citrate-phosphate buffer 25 mM (pH 6.0) • Purified protease was g glycine-sodium hydroxide buffer 100 mM (pH 9.0) at 70 °C,	• Anti nematicidal against M. incognita juveniles
Protease (Application 2015)	E.trigona, E.maculata, E. milii var imperata	• Fresh latex was cooled (at -20°Cfor 24 hours) and centrifuged (24,000 × g for 45 min) • Spectrophotometry with 595nm with Bradford assay and BSA standard was used for protein concentration determination.	• Milk clot formation

2.2.3. Latex Coagulation

Plant latex is excluded from the plant and exposed to air it coagulates immediately. Normally latex is a kind of chemical process and is also affected by physical factors like particle size, humidity, temperature and also differs from species to species (Wahler et al. 2009a). At normal pH conditions, all components of the latex that is the solids pellet-like plastids known as Lutodies, together with the specialized plastids or Frey-Wysslings complexes and clear C-serum rubber supernatant having all secondary metabolite's and all types of proteins are found to be stable without any interaction. When the pH of the system is around 4.00 and less the isoelectric interaction happens and coagulation takes place (I, Khan, and Akhtar 2003). The research direction that reports the coagulation of the plant latex as a result of pH change discusses based on bacterial entrance to the latex when it is exposed to air, then producing lactic acid when hydrolyzing lipid-like components in the latex system. As the lactic acid keeps being generated in the system the positive hydronium ions interact with the negative charges that surround the membrane of the latex hence the particle start to collide resulting the burst of the surrounding membrane and interaction of rubber (isoprene units).

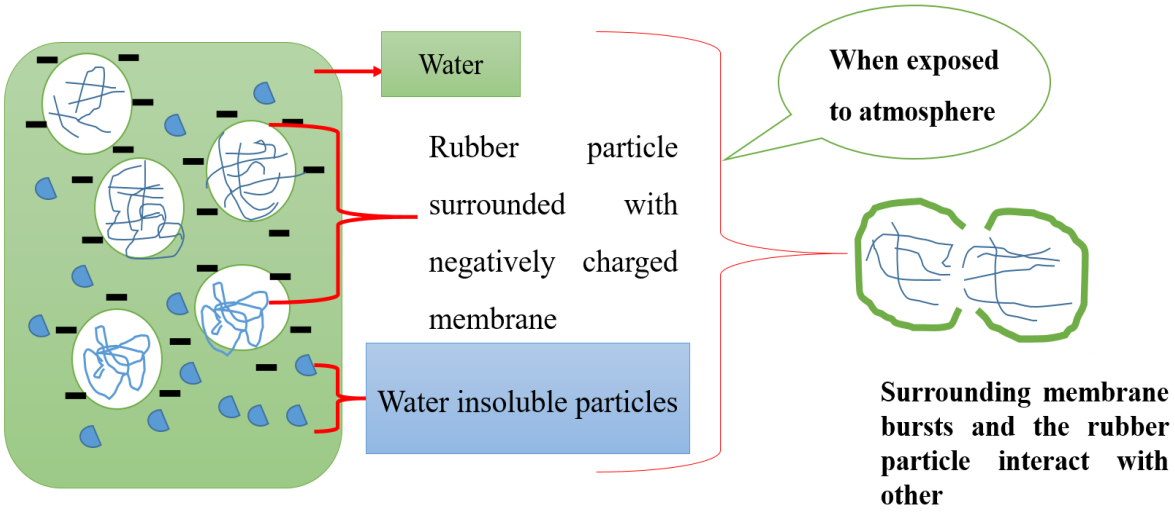


Figure 2. 5: Coagulation process of plant latex

The coagulation process is believed to be the result of enzymes in the latex systems like Heva lectin binding, chitinases, (Wititsuwannakul et al. 2008) where others suggest the stability of the colloidal is the result of the negatively charged latex particles that undergo continuous

repulsion(Agrawal and Konno 2009). More generally considering rubbery latex's the coagulation process can be introduced to have different stages that are equilibrium state, laticifier rupture, apoplastic transport, and plug formation. Chitinases do not interact with glucanase under normal conditions. However, when a laticifier is wounded or damaged mechanically, fresh latex flows out and the turgor pressure drops significantly. The Lutodies burst as a result of the decrease in pressure. As a result, the membrane-embedded hevamine interacts with membrane-localized glucanase, facilitating the aggregation of rubber particles and subsequent rubber latex coagulation. Finally, latex vessels become blocked up with rubber coagulates, resulting in the stoppage of latex flow (Bioactive and Derivatives 2016)(Salomé Abarca, Klinkhamer, and Choi 2019).

The final stage of the latex coagulation results in a polymer-like material having an irreversible state, and it is a big challenge because it limits the commercialization of the latex so this mode of action and also preventions as a means of preserving the liquid milk latex is extensively studied by the rubber technologists. Some methods are reported for the preservation of plant latex including additions of anticoagulants to adjust the pH of the latex. The well-tried chemicals used as anti-coagulant of the plant latex are glutaraldehyde, paraformaldehyde, potassium permanganate, phosphate buffer (Fineran 1989), and ammonia solution which is the short term preservation and stabilizes the system by hydrolyzing the free fatty acids and the lipids in the system other than increasing of the pH of the system (Azadi et al. 2020). Other than preserving the plant latex in its liquid form different works can be done that involve the respective use of plant latex for the required purpose. These include extraction of bioactive compounds for medicinal purposes, extracting rubber from it using different extraction methods. Commercialized natural rubber is known to extract from the well-known Euphorbiacea rubber bearing plant where experimental works are being reported as sources of natural rubber. Modification of the liquid latex tapped from the plant and using it for different, purpose is also the other trend.

2.2.4. Plant Latex and its Irritating Nature

The milky-like substance excluded from many plants especially from Euphorbiaceae species is reported to be toxic. Although available reports show that Euphorbia sap is irritant to the skin, the exact mechanism underlying these inflammatory reactions has not yet been determined but researches are being done to identify those toxic substances. One possible mechanism is that phorbol esters in the plant's sap have a direct corrosive effect with direct pH-induced cytotoxicity,

resulting in necrotic damage to keratinocytes. Ingenol mebutate can enhance these effects by inducing a pro-inflammatory environment and cell death(Weber et al. 2019). Fingerprinting of toxicity makers was performed using chromatography techniques on five different Euphorbia species namely E.ammak, E.clavarioides, E.caerulescens, E.polygona, and E.trigona and found the method used for the investigation was effective for identification of toxicity makers that is TLC combined with preliminary phytochemical screening. The protein test gave a negative test for all plants with both well-known standard test methods whereas the other phytochemicals that are used as a bioactive component were found to be present (Mampa, Mashele, and Sekhoacha 2020).

The irritant nature of plant latex is due to the presence of polycyclic diterpenes esters that are irritant to insects and herbivores also (Bigoniya, Shukla, and Singh 2010). The research result from this group involves screening of the toxic component making that is polycyclic diterpenes from the latex of *Euphorbia nerifoliol* and promoting topical use the well-known bioactive component like triterpene and steroid. According to the Draize Dermal Classification System, ether fraction was found to be nonirritating, with a PII score of 0.43/0.11 for erythema and edema. Because of the presence of high diterpenes content, chloroform, acetone, and water fractions are skin irritating, whereas petroleum ether was non-irritating. The ether fraction is high in triterpenes, which have nonirritant activity. Anti-inflammatory activity of latex petroleum ether fraction is due to the presence of triterpenes euphol, nerifoliol, and cycloartenol. The other research group (Fürstenberger and Hecker 1985) also isolate the chemical and toxicological properties of the irritant constituents of *Euphorbia triculi L*. the acetone extract of the plant latex yields two types of fractions the hydrophobic having the non-irritant parts and the hydrophilic part containing irritant component. The hydrophilic fraction was subjected to further purification to give a non-irritant biologically active component. The chemical structure of the isolated components was investigated by analytical instruments like NMR, mass chromatography, UV, and their irritating nature was found to result from the acetic acid and long-chain carboxylic acids carrying carbonyl conjugated double bonds having UV maxima between 267 and 356 nm.

The tumor-promoting activity of esters decreases as the number of C = C bonds in the acyl chain increases, implying that selective transesterification with $NaOCH_3/CH_3OH$ removes the acetyl group. Aside from purification and modification to overcome the toxic nature, this research group discovered that the presence of those components varies depending on the location and climatic

conditions in which they are found. Similar research reports were done, for another Euphorbiacea species *Euphorbia Pepulus* having the more unsaturated hydrocarbon components that have a contribution to the irritating nature of the latex (Giner, Berkowitz, and Andersson 2000).

2.3. Terpene Based Bio-Conjugates

Terpenes are natural compounds found in a variety of organisms from the animal and plant kingdoms. They are the most diverse class of natural products, with over 55,000 known structurally diverse compounds. Several studies have linked this large family of compounds to a variety of pharmacological properties, including anticancer, antimicrobial, antifungal, antiviral, ant-hyperglycemic, and analgesic properties (Serafini and Guimara 2014). While preserving the bioactivity of the terpene compounds conjugation can be performed to drive other parts of those chemicals. This derivation may be either by using cross-linker or by Lego chemical concept in which n a few steps, of molecular synthesis was as simple as building a Lego construction from single blocks and readily available structure units could be easily linked together to final form made of final molecules was produced (Pawełczyk et al. 2020).

New triterpene derivatives can be synthesized by reacting phenoxy acetic acid commonly known as triterpenes (oleanolic acid/oxime) (Pawełczyk et al. 2020). The synthesis method can follow a two-stage generation of phenoxy acetic acid with micro-wave-ultrasonic (MW-US) assisted O-alkylation system together with using and then reacting with the triterpenes having an active hydroxyl group using halogen acidic derived as a cross linker. With this synthesis method, almost 8 derivatives of triterpene were developed with an effective bio-activity characterized by the drug score method. The reaction of the secondary hydroxyl group or hydroxyl-imino group that results from it, present in the terpene molecule at C-3 position with a free carboxylic moiety of phenoxy acetic agents is the main transformation that yields linked derivatives. 1:1.5:5 (oleanolic acid oxime, functionalized phenol, DCC) molar ratio was used and reactions, both towards ester and imino-ester-type compounds, were carried out at room temperature and in mild conditions with anhydrous dimethylsulfoxide was as a solvent used. Similar research reports are available towards the conjugate formation of terpene compounds (mostly triterpene) that focus on their bio-activity nature. The bio-activity of the natural products is related to their chemical structure which needs to be preserved while modifying.

Most of the terpene-based compounds especially the Penta-cyclic ones are known to be non-polar hence insoluble in water. When this phenomenon happens in the field of medicinal application it will be a drawback that restricts the use of those bioactive compounds for the synthesis of drugs. So to overcome this challenge sugar moieties are inserted at the hydroxyl, carbon to carbon double, or via the carboxylic groups of the terpene skeletal. Synthesis of water-soluble pentacyclic triterpene can be achieved via attachment of ester bond with classical method or via amide bond attachment. This synthesis method can follow a novel method of forming the desired product the application in the area of medicine. The derivatives showed an acceptable cytotoxicity effect towards the test sample confirming the modification of pentacyclic triterpenes can be controlled as not to lose their bioactivity (Jiao et al. 2019). This can confirm that those naturally available compounds (terpenes) can undergo conjugation with their hydroxyl group, carbon to carbon double, or the carboxylic group attached to their skeletal. Simultaneous availability of those groups is not a must to undergo the modification when necessary, with the classic method using an appropriate linker is also possible (H. Wang et al. 2016).

Other than their application in medicine terpene conjugate have industrial application in which the synthesis method may differ based on the application. Directly polymerized terpenes are mostly monoterpenes like myrcene, pinene, and limonen, β-myrcene (7-methyl-3-methylene-octa-1, 6-diene) as an acyclic monoterpene can polymerize to give a thermosetting structural adhesive with sulfur dioxide and Azobisisobutyrate as an initiator. The reaction temperature can be 70°C with a reaction time of 30 hours. The reaction temperature higher than 70°C results in degradation of the initiator and homo-polymerization of the monoterpene appears in turn. The synthesized copolymer exhibited dry strength of 4.42 ± 0.28 Nmm^{-2} and a wet strength of 1.74 ± 0.37 Nmm^{-2} adhesive strength on wood substrate. The effectiveness of the adhesion nature on the wood substrate is due to the bonging nature of the hydrogen bonding between the adhesive and the wood surface, it exhibits lower strength for wet support that is because the water restricts the bonding process in between (Guo et al. 2019).

The transformation of terpenes towards ester is through the condensation reaction of the carboxylic group in the skeletal. Other than this several polymerization methods can be used with various terpenes like radical, ionic, or even coordinative polymerizations of monomeric terpenes (Sahu, Bhowmick, and Kali 2020). The β-pinene a camphor-based lactam can be transformed to poly β-

pinene via anionic ring-opening polymerization (ROP) using sodium hydride and KOtBu as an initiator. The temperature and an equal molar ratio of reactants and co-initiator give a polyamide polymer having thermal property comparable to the commercial Nylon-6 polymer confirming successful polymerization of terpene monomer via ring-opening (Winnacker and Sag 2018).

2.4. Lactic acid: Its Application and Bio-compatibility

Lactic acid is a three-carbon organic acid found in many natural products, with one terminal carbon atom belonging to an acid or carboxyl group, the other terminal carbon atom connected to a or hydrocarbon group, and a central carbon atom connected to an alcohol carbon group. It is also known to be an alpha hydroxyl acid due to the hydroxyl group attached to the carboxyl group. Lactic acid exists in two optically active isomers. The first is L-lactic acid, also known as (s)-lactic acid, and the second is D-lactic acid, also known as (r)-lactic acid.

L-lactic acid D-lactic acid

Figure 2. 6: Two isomers of lactic acid

The first reports on the isolation of lactic acid from milk were found in early 1780 and the solidification by self-esterification some years later. Lactic acid can be manufactured by chemical synthesis or carbohydrate fermentation or purified either by precipitation of metal lactates followed by a neutralizing reaction with sulfuric acid or esterified with alcohol, distillation and hydrolysis of the formed ester or by electro-dialysis (Hamad et al. 2018)

A poly-condensation process that involves the linking of LA monomers together through reactions between the -OH and -COOH groups and the removal of byproducts such as water and alcohols from the reaction system can convert LA monomer to PLA. Because the viscosity of the mixture increases significantly during this process, water removal becomes more difficult. Because the viscosity of the mixture increases significantly during this process, water removal becomes more difficult, resulting in the formation of a low-molecular-weight product. Thus, the poly-

condensation of LA monomers can be divided into two distinct methods, depending on whether or not an organic solvent is used to dissolve the PLA (Hamad et al. 2018).

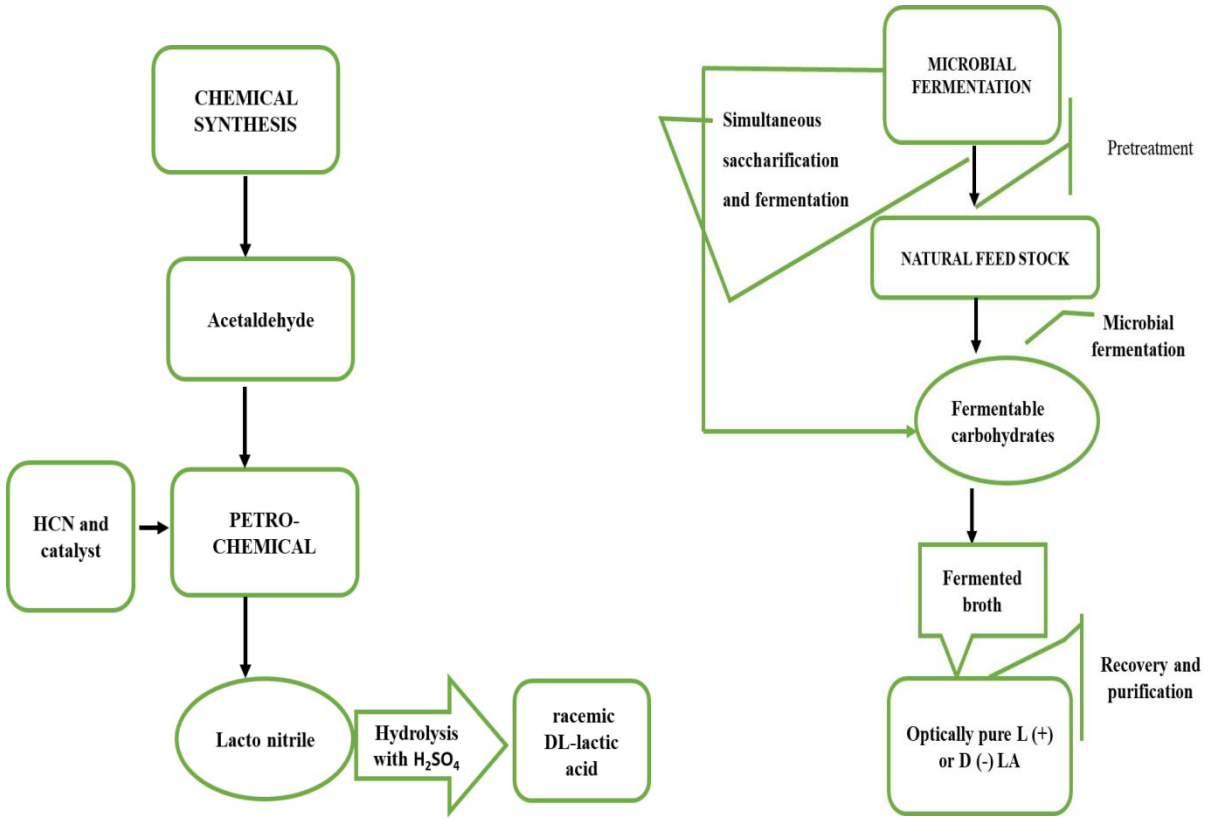


Figure 2. 7:The two common methods for the production of lactic acid (Komesu et al. 2017)

From widely known commercial methods fermentation of polysaccharides is one of the chemical synthesis methods for lactic acid production (Narayanan 2004). This method produces a racemic mixture of lactic acid using hydrogen cyanide, acetaldehyde, and the presence of a base to produce lacto-nitrile, which is then purified by distillation and hydrolyzed to lactic acid, either by concentrated by H_2SO_4 to produce the corresponding ammonium salt and lactic acid. This research team suggests the ring-opening polymerization of the synthesized lactic acid for the production of polylactic acid. Poly-condensation of lactic acid is followed by polymerization into the dehydrated cyclic dimmer, lactide, which can be ring-opening polymerized to high molar mass polymers. Depolymerisation is traditionally accomplished by raising the poly-condensation temperature and decreasing the pressure, followed by distillation of the resulting lactide (Narayanan 2004). In the other studies, L-Lactic acid was then polymerized to form PLA with the azeotropic polycondensation method was used in the polymerization process. The reaction is a modification

of a traditional polycondensation reaction that can produce a higher molecular weight (Series and Science 2021). The yield of PLA was found to increase with increasing mass of starting material i.e., lactic acid. At a technical lactic acid value of 40 grams, the best-operating conditions for technical lactic acid polymerization have been obtained. Xylene solvent up to 100 grams, 0.5% $SnCl_2$ catalyst and a 30 hour polymerization time yielded a PLA yield of 49.6%, a molecular weight of 57.066.9 g/mol, and a melting temperature of 120°C. By varying the reaction duration and the sequence of catalyst addition, high molecular weight PLA can be produced also (Singh, Anthony, and Chowdhury 2018). The binary catalysts used were $SnCl_2.2H_2O$/maleic anhydride and p-toluene sulfonic acid and PLA with molecular weight (6.503X105) was obtained in 52 hours.

Ring-opening to form poly-lactic acid is more favorable than the poly-condensation of lactic acid to give poly-lactic acid polymer because the polymer from the poly-condensation is found to be low in molecular weight. Controlling the equilibrium between the water removed, the lactic acid, and the poly-lactic acid is one way of using the ring-opening mechanism easier (Narayanan 2004). The ROP method is a cyclic monomer propagation process. Because the process can be initiated by ions, the mechanisms of anionic, cationic, and radical ROPs are classified. In the case of PLA, the cyclic monomer is the lactide, which is the cyclic dimer of lactic acid. To achieve controlled molecular mass, appropriate metal catalysts were used, and the temperature is regulated (Horváth, Marossy, and Szabó 2021). The polymeric material from this study was characterized with different analytical instruments like FT IR, GPC and found to have a comparable and similar property to commercial PLA.

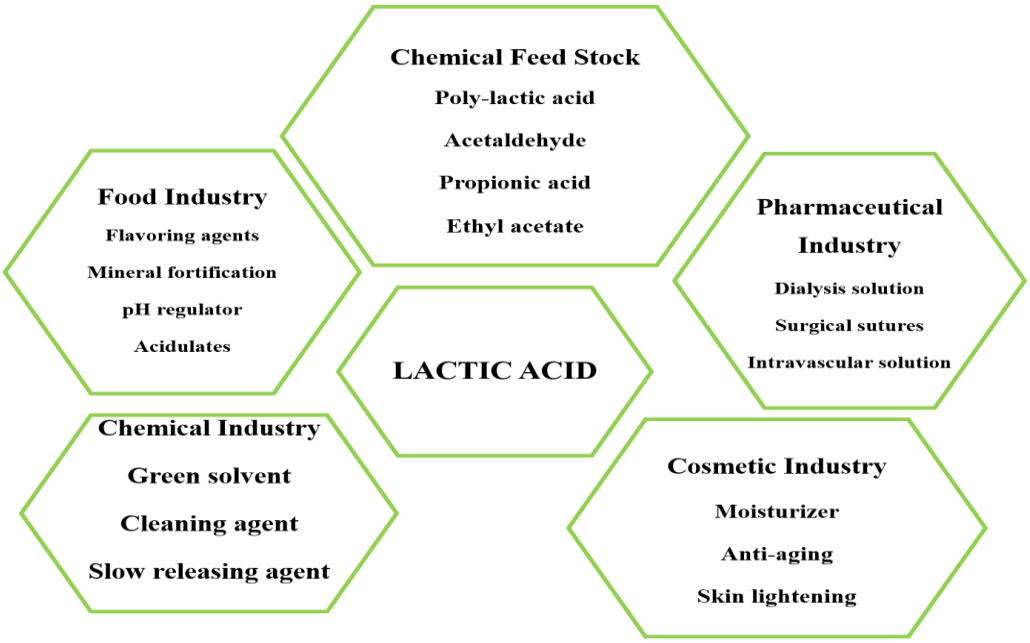


Figure 2. 8: Summarized application areas of lactic acid (Ahmad, Banat, and Taher 2020)

2.5. Bio-Based Adhesives in Biomedical Application

The most widely used medical adhesive is cyanoacrylate which is also known as super glue and has strong adhesion, rapid setting time, instantaneous adhesion to tissue, and ease of use with simple preparation making it to be properly applied for clinical uses and are widely used in emergency rooms, dermatology, and plastic surgery (Mehdizadeh and Yang 2013). Besides its well-accepted advantages, there exist some drawbacks that restrict it from using it in topical skin. Those are exothermic reactions, i.e., heat generation during polymerization, and concerns about the toxicity of degradation products, lack of flexibility for soft tissue adhesion, difficulties inaccurate delivery due to its low viscosity, weak shear strength of joint area especially in the presence of water, high stiffness that can cause undesired consequence such as adhesion failure and tissue irritation, and infection due to existence of non-absorbable polymer. These drawbacks were tried to be solved by the specialists under the area through addressing absorbable adhesive polymers using more hydrophilic cyanoacrylates, which comprise, using of plasticizers, stabilizers, accelerators, viscosity-adjustment agents (Bhagat and Becker 2017). The other synthetic adhesive used in the biomedical adhesive used as a tissue sealant is polyethylene Glycol (PEG) which has rapid gel formation, adhesion to the biological surface, the biocompatibility of polymer, and degradation products and inducing mild to a moderate inflammatory response. Over

its bio-compatibility and good performance, there is a concern related to its swell ratio of up to 400% of the original volume, which necessitates greater caution when used in enclosed spaces to avoid pressure buildup on surrounding tissues (e.g., nerve compression). Furthermore, it requires a relatively dry surface for best results. So, some of the researchers do their work towards improving the drawback of those synthetic adhesives while the other move towards bio-based adhesives and sealants through using different modification techniques, like grafting and conjugate development (Chen et al. 2018).

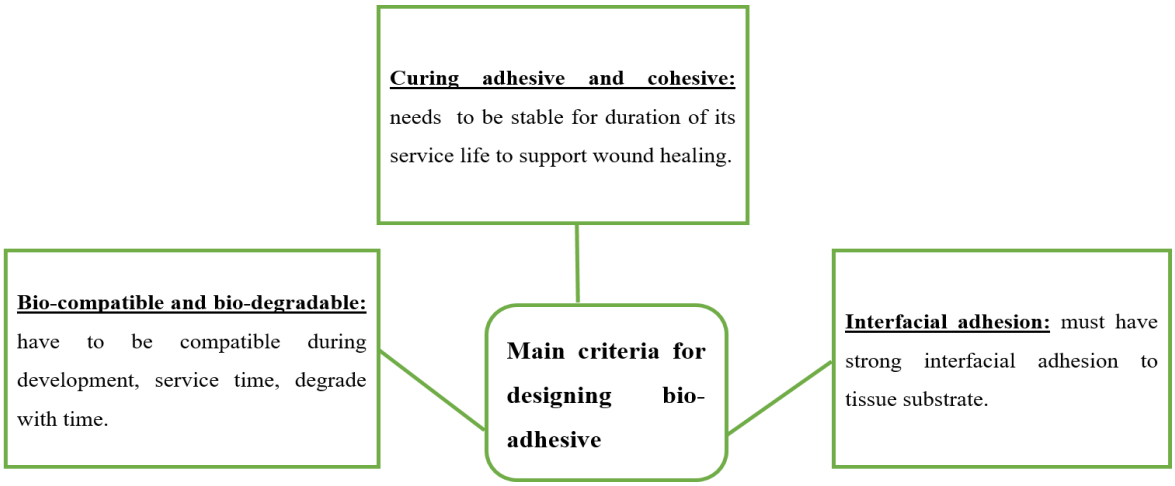


Figure 2. 9: Main criteria needed to be fulfilled during designing a bio-adhesive for biomedical application (Pinnaratip et al. 2019).

A conjugated system in chemistry is a system of connected p orbitals with delocalized electrons in a molecule that reduces the overall energy of the molecule and increases stability. It is typically represented by alternating single and multiple bonds. The system, which can be cyclic, acyclic, linear, or mixed, may contain lone pairs, radicals, or carbenium ions. More generally conjugate refers to a compound formed by the joining of two or more chemical compounds. Those conjugated materials are termed bio-based, basically when they are derived from animals, plants, and micro-organisms as starting material (Gu et al. 2019). As it is known naturally occurring biopolymers lack some kind of performance hence need modifications through grafting an oligomeric or polymeric core, with either the arms, the core, or both being conjugated hence synthesizing grafted conjugated copolymers. The synthesis of graft conjugated systems is possibly the most diverse of the three types of 3D-structured conjugated polymers described, as it includes methods for preparing star-shaped (mainly focusing on linking arms and cores-grafting “onto”) and

dendrimeric (mainly functionalization and branching at active sites-grafting “from”) systems, as well as a variety of other methods including reactive assembly of the core through the polymerization of macromolecular monomers by grafting “through” (Jarosz, Gebka, and Stolarczyk 2019). These conjugated polymers after the proper modification have a different application or can be synthesized based on the predetermined application areas which include biomedical (Sahana and Rekha 2018) as the substituent for different tissues or as a drug delivery system (Winnacker and Sag 2018), as adsorbent (Ramakrishnan et al. 2021), even in the photo engineering system (Anguera et al. 2014)(Tadesse 2018). Naturally occurring biopolymers and polysaccharides can undergo similar modifications with different conjugation schemes and be used as adhesive also for various applications including medical adhesives which have both antimicrobial property and the adhesion nature together (Guo et al. 2019). The well utilized and broadly studied ones include proteins which possess both adhesion nature and anti-microbial activity that facilitates the bodies well beings especially for wound healing purpose (Al-salami 2021). This research group synthesized a bio adhesive polymer prepared by modification of pepsin structure with vinyl monomer such as maleic anhydride using ceric ammonium nitrate as an initiator, then the grafted copolymer was substituted with amino drugs. The graft copolymerization was confirmed by FT IR and NMR analysis where the drug release in vitro was studied with different pH values at 37ºC with UV spectroscopy and further confirmed by the in vivo test which resulted to be a promising substituent for applications of polymer used different mice and rabbits infected with bacteria and wounds.

2.6. Research Trends towards Biomaterials

As biomaterials combination of substances other than drugs, synthetic or natural, which can be used for any period of time and augments or replace any tissue, organ, or function of the body to maintain or improve the quality of life of the individual. Due to their availability, especially their bio-compatibility towards the environment researchers from both academic and research and development centers of the industries are intensively working on bio-materials (Tathe, Ghodke, and Nikalje 2010).

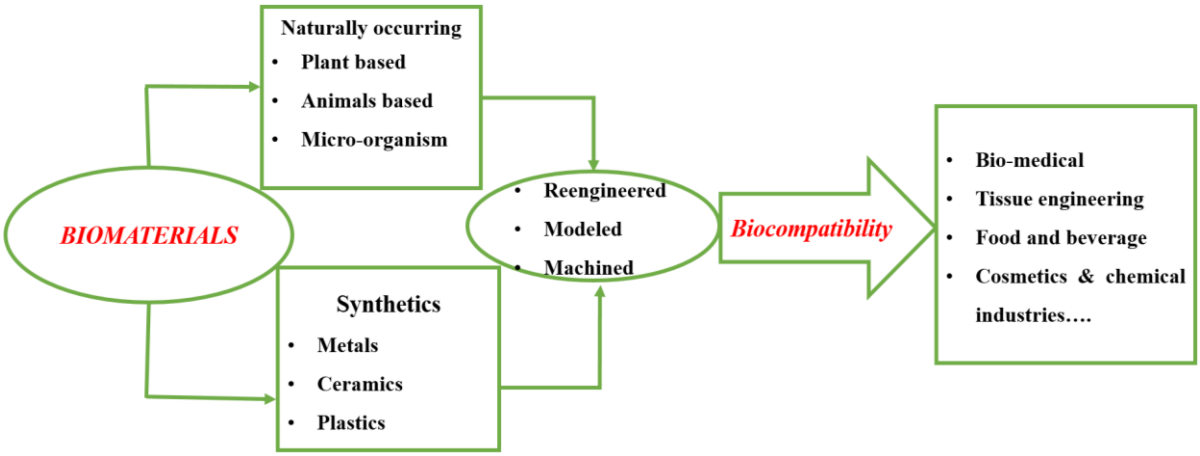


Figure 2. 10:Major sources of biomaterials and representative application areas (Hossein et al. 2018)

Natural products are defined on the basis of their source as the name itself indicates, including materials such as animals, marine organisms, plants/botanicals/herbals, macroscopic fungi, bacteria, probiotics, and minerals, and materials derived from them, including isolates, extracts, vitamins, and amino acids. Those materials may vary in chemical complexity, ranging from crude to extensively purified materials, and were considered to include dietary supplements, certain (Ige, Umoru, and Aribo 2012).

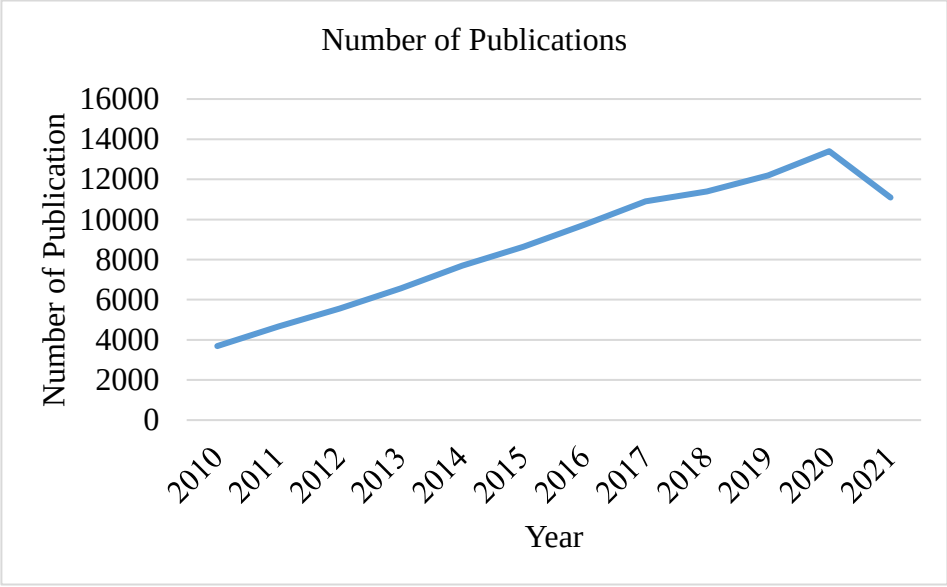


Figure 2. 11: General research trends on biomaterials through number of publications up to August 28, 2021.

CHAPTER 3

3. MATERIALS AND METHODS

3.1. Laboratory Materials and Chemicals Used Under the Study

L-lactic acid (88% aqueous solution), petroleum ether, ethanol (99%), with laboratory grades were purchased from the local market. The other chemicals were used from the laboratory and they are summarized in the following table (Table 3.1).

Table 3. 1: Summary of materials and chemicals used under the study

Chemicals	Materials	Purpose
Buffer solution, ethanol, distilled water	pH meter, beakers, latex, balance, digital visco-meter, petri dish, crucible, furnace, oven	Physico-chemical characterization of latex
Distilled water, acetone, $CHCl_3$, petroleum ether	Beaker measuring cylinder, glass sample holder, ETM latex	Solubility study of ETM latex
Mayer's reagent, NaOH, H_2SO_4, $FeCl_3$, $CuSO_4$, NaOH	ETM latex, measuring cylinder, balance	Preliminary phytochemical screening
Lactic acid	ETM latex, RBF, measuring cylinder, horizontal condenser, oven,	Synthesis of ETM-*l*-OLLA bio-conjugate
$CHCl_3$, KOH solution, phenophitalin,	Conical flask, pipete, measuring cylinder, balance, bio-adhesive	Acid value determination
DMSO, distilled water	Petri dishes, ETM-*l*-OLLA, ETM latex, OLLA	Anti-bacterial study

3.2. Methods

3.2.1. Raw Material Collection

The main raw materials used for the synthesis of the bio-adhesive that is *Euphorbia trigona* Mill latex was collected from Adama city starting from August 9, 2021. The *Euphorbia trigona* Mill tree was cut by knife and the latex was collected in a clean sample holder. The large impurities like tree parts were removed by decanting and stored in an air-tight container in a refrigerator until further use.

3.2.2. Physico Chemical Characterization of *Euphorbia trigona* Mill latex

Physico-chemical properties like pH, density, dynamic viscosity, and moisture content, ash content, and total solid content, total resinous matter were investigated for the *Euphorbia trigona* Mill latex. All the experiments were performed three times for better precision and the average value was reported.

pH determination (ASTM E70): The pH value of the latex was measured according to the ASTM E70 procedure using a digital pH meter. Before starting the measurement the pH meter was calibrated to the value of 7 using buffer solution. 10 mL of freshly trapped *Euphorbia trigona* Mill latex was taken and put into a pre-cleaned 50 mL beaker. The pH electrode was immersed into the *Euphorbia trigona* Mill latex sample. Then waited until a constant reading was observed and the value was taken from the controller display.

Figure 3. 1: pH determination of *Euphorbia trigona* Mill latex with a digital pH meter

Density determination (ASTM D1076): The density of freshly trapped ETM latex was determined according to ASTM D1076 using a metal density cup with a capacity of 100mL. The

pre-cleaned density cup was weighted using digital weighing balance and its weight was recorded as W1. 100 mL of freshly trapped ETM latex was added to the density cup and its weight was measured using digital weighing balance and recorded as W2. Then the density of the latex was calculated using the following formula,

$$\text{Density} = \frac{W2 - W1}{V} \qquad \text{Eq 3. 1}$$

Where: V is the volume of latex

Figure 3. 2: Determination of density for freshly trapped *Euphorbia trigona* Mill latex

Dynamic viscosity determination (ASTM D 1084): The dynamic viscosity of freshly trapped ETM latex was measured using digital VK - 2000 MYRS KREBS viscometer according to ASTM D 1084. 200 ml of freshly trapped ETM latex was placed into a pre-cleaned beaker with a capacity of 250mL. Then, the vibrio viscometer tip was inserted into the latex sample to measure the dynamic viscosity and the reading was taken from the controller display.

Figure 3. 3: Determination of Viscosity of *Euphorbia Trigona Mill* latex with digital viscometer

Determination of moisture content (ASTM D2216-19): The moisture content of ETM latex was determined according to ASTM D2216-19. Glass Petri dishes were washed, dried, and then 20gm ETM latex was poured on and dried in a drying oven for 6 hours until a constant weight was maintained. The moisture was calculated as the difference between mass before drying and after drying.

$$\% \text{ Moisture content} = \frac{W1 - W2}{W1} \qquad \text{Eq.3. 2}$$

Where W_1 is the weight of the ETM latex samples, W_2 is the weight of the ETM latex samples after drying.

Determination of ash content (ASTM D2584): The ash content of the ETM latex was determined according to ASTM D2584. An oven-dried sample was weighted on a crucible dish and put in an electrical muffle furnace at a temperature of 500 ºC for 1 hour. Then the ash content was determined with the following formula.

$$\text{Ash} = \frac{W1-W2}{W1} \times 100\% \qquad \text{Eq.3. 3}$$

Where; W_1 is the dry weight of the moisture-free sample, W_2 is the weight of the sample after ignition

Total solid content determination: The total solids content of ETM latex was estimated based on the method reported (Bigoniya, Shukla, and Singh 2010). 5mL of ETM latex was poured in a

precleared boron silicate glass petri dish and put in a drying oven at a temperature of 110 ºC for almost 5 hours until a constant mass was obtained. The total solid content was calculated with the following formula.

$$\text{TSC} = \frac{\text{Initial mass of latex}}{\text{Final mass after oven drying}} \times 100 \qquad \text{Eq.3. 4}$$

Estimation of the total resinous matter: The total resinous matter of ETM latex was estimated based on a method reported by [26]. 10mL of freshly trapped ETM latex was treated with 20mL of pure ethanol (99%) three times and the ethanol-soluble part was decanted. In the concentrated ethanol fraction 25mL of distilled water was added white flocculates were formed it was decanted dried in an oven at 105ºC to constant weight. Then the total resinous matter was estimated in the following formula;

$$\text{TRM} = \frac{\text{Initial weight of latex before oven drying}}{\text{Final weight after drying}} \times 100\% \qquad \text{Eq.3. 5}$$

Solubility study: The solubility of the ETM latex was studied based on a method reported by [26]. The solubility nature of the latex after curing at room temperature and fresh latex was studied by dissolving in chloroform, acetone, petroleum ether, and water. The know amount of dried latex was dissolved in each solvent with a 1:2 weight to volume ratio and waited for 12 hours with occasional shaking. Similarly, the freshly trapped ETM latex was studied for the same solvent.

3.2.3. Investigation of Preliminary Phytochemical Screening of ETM latex

Freshly trapped ETM latex was analyzed for the common phytochemicals. The preliminary phytochemical test was performed based on standard test methods.

Test for alkaloids: 1 mL of the ETM latex was transferred in a test tube with the help of a dropper and three drops of Mayer's reagent were added dropwise. The formation of a white or pale yellow precipitate indicates the presence of alkaloids.

Test for tannins: 1 mL of ETM latex was transferred in a test tube with the help of a dropper and 2 ml of 5% ferric chloride was added. The formation of dark-blue or greenish-black color shows the presence of tannins.

Test for flavonoids: mL of ETM latex was transferred in a test tube with the help of a dropper. 5 ml of dilute ammonia solution was added into it, followed by the addition of concentrated H_2SO_4. Yellow color will be observed showing the presence of flavonoids. The yellow coloration disappeared on standing.

Test for saponins: 3mL of ETM latex was transferred in a test tube with the help of a dropper. 10 mL of distilled water was mixed into it. The test tube was stoppered and shaken vigorously for about 5 min, then honeycomb froth was formed and it was allowed to stand for 15 minutes. The formation of a stable honeycomb forth shows the presence of saponins.

Test for terpenoids: 1mL of ETM latex was transferred in a test tube with the help of a dropper and treated with 2 ml of chloroform and concentrated sulphuric acid. The formation of red-brown color at the interface indicates the presence of terpenoids.

Test for phenols: 1mL of ETM latex was transferred in a test tube with the help of a dropper. 2 ml of distilled water followed by a few drops of 10% ferric chloride was added to 1mL of latex sample. The formation of blue or green indicates the presence of phenols.

Test for cardiac glycosides: 0.5 ml ETM latex was transferred in a test tube with the help of a dropper. 2 ml of glacial acetic acid and a few drops of ferric chloride were added, followed by 1 ml of concentrated sulphuric acid under layer. The presence of cardiac glycosides is indicated by the formation of a brown ring at the interface.

Test for anthraquinone glycosides: 1 ml ETM latex was transferred in a test tube with the help of a dropper followed by a few drops of 10% ammonia solution. Pink color precipitate indicates the presence of anthraquinone glycosides.

Thin Layer Chromatography: For the TLC analysis silica gel on thin aluminum plates (5×10 cm) was used as stationary phase. For the mobile hexane: ethyl acetate (7:3), toluene: chloroform: acetone (4:2:3), DCM: methanol (6:1) were used. The freshly trapped ETM latex was spotted on the TLC plates using the capillary tubes. Then the plates were dipped in the prepared TLC chamber with the respective solvent system let to develop and the developed TLC plates were visualized under the UV light a wavelength of 254nm and 365nm. Then the distance moved by the spot and also that of the solvent (in *Mill*imeter) was measured with a ruler and used to determine the retention factor with the following formula; equation 3.6,

$$RF = \frac{\text{Distance traveled by spot(mm)}}{\text{Distance traveled by solvent(mm)}} \qquad \text{Eqn. 3. 6}$$

3.2.4. Functional Group Analysis

The functional groups available in freshly trapped ETM latex were determined using Fourier transform spectroscopy (FTIR). Fourier transform spectrophotometer measured on Spectrum 65 FT-IR (PerkinElmer) in the range 4000-400cm-1 (resolution 4cm-1, number of scans 4) using KBr pellets. This experiment was done at Addis Ababa University, college of natural science department of chemistry

3.2.5. Analysis of Chemical Structure

The chemical structure of freshly trapped ETM latex was analyzed with Nuclear magnetic resonance spectroscopy. The sample was dissolved in deuterated chloroform (CDCl3) and filtered with a 0.25 μm filter and transferred to a 5 mm diameter NMR tube. Similar to the FTIR analysis this experiment was done at Addis Ababa University, college of natural science department of chemistry.

3.2.6. Thermal Property Analysis of *Euphorbia trigona* Mill Latex

The freshly trapped ETM latex and also air-dried ETM latex were performed to investigate the difference between liquid and solid ETM latex. The freshly trapped ETM latex was shade dried and reduced in size. Samples were (both liquid and solid) heated from 20°C to 800 °C at the heating rates of 20°C/min under nitrogen (N_2) atmosphere.

3.3. Synthesis of *Euphorbia trigona* Mill Latex –Lactic Acid Adhesive

The *Euphorbia trigona* Mill latex–lactic acid adhesive was developed based on a method reported by (Tripathi and Katiyar 2018). The ETM latex-lactic acid bio-adhesive was prepared by in-situ polycondensation reaction of L-lactic acid (88%) and latex. Mix ratio, reaction time, and reaction temperature were selected based on preliminary experiments. With a method of keeping one variable at a time the visual observation, physical tests, and titration were used to select the suitable reaction condition for reaction time. Specifically, different varying amounts of latex (25,50,75 V%/V%) were taken into a round bottom flask with a capacity of 500mL and mixed with a magnetic stirrer for proper dissolution of the latex and lactic acid. Then the round bottom flask was put in a reactor with a controlled temperature system. The horizontal condenser was connected

to the round bottom flask neck with the help of an elbow connecter. And chiller was connected to circulate water and facilitate withdrawal of byproduct water from the reaction and the reaction was let to continue 3hr. The byproduct of water was then collected in a beaker from the other end of a condenser. The bio-adhesive was synthesized at the reaction time completed and the resulting product was collected and kept in a closed sample holder for further analysis each experimental run. The experimental setup used for the synthesis of the bio-adhesive can be shown in the following figure (figure 3.4). The synthesized bio-adhesive systems with varying amounts of ETM latex were then labeled ETM-*l*-OLLA 1, ETM-*l*-OLLA 2, and ETM-*l*-OLLA 3 indicating the mix ratio of 1:1, 1:2, and 1:3 of lactic acid to ETM latex. As a control system lactic acid oligomer was synthesized with a similar setup to that of bio-adhesive development.

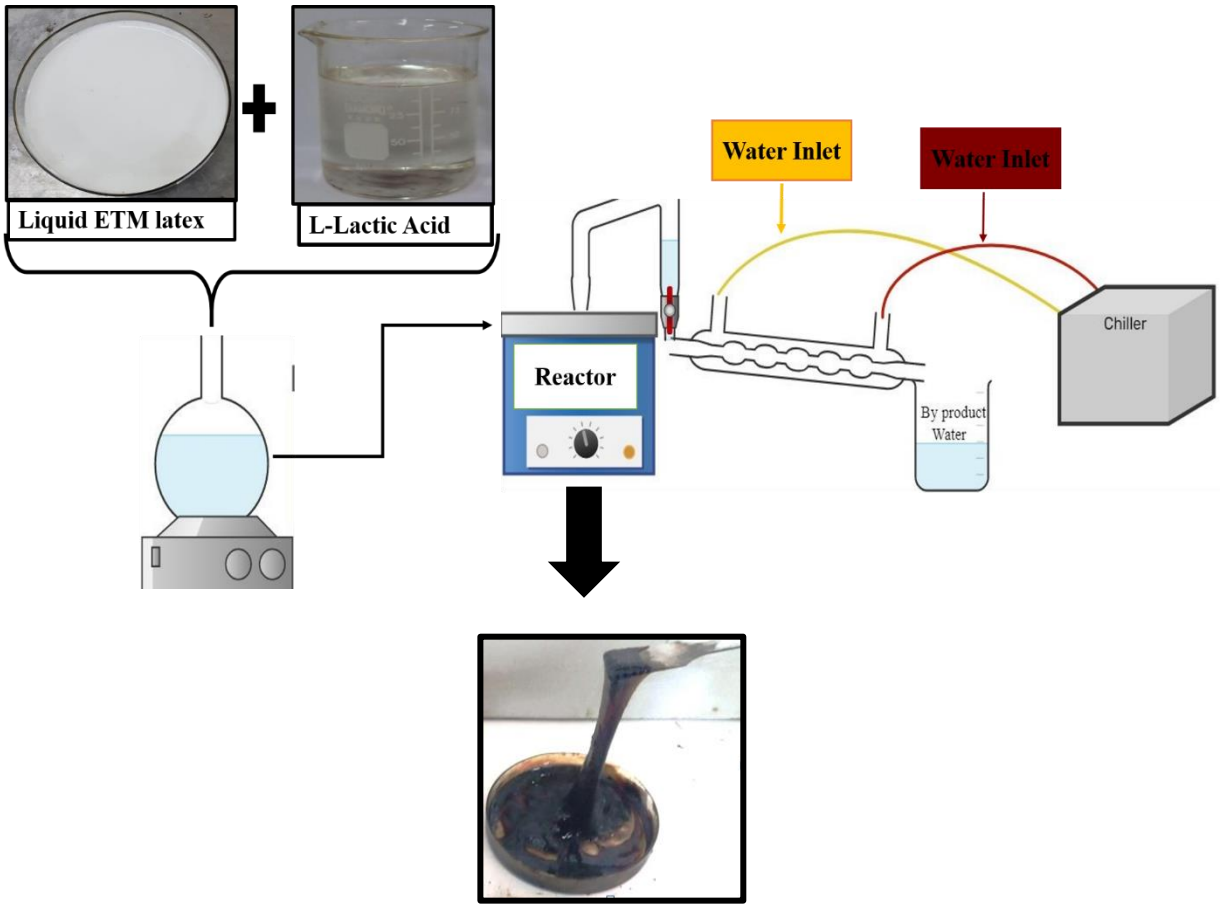


Figure 3. 4: Reaction setup for the synthesis of ETM-*l*-OLLA bio-adhesive.

The reaction condition that is chosen based on the preliminary experiments can be summarized in the following table.

Table 3. 2: Reaction condition for the synthesis of ETM-*l*-OLLA bio-adhesive

Rxn time (hr)	Rxn Temperature (°C)	Mix ratio	Product
For 3 hours	At 150°C	1	ETM-*l*-OLLA 1
		2	ETM-*l*-OLLA 2
		3	ETM-*l*-OLLA 3

3.3.1. Characterization of *Euphorbia trigona* Mill Latex-Lactic Acid Bio-adhesive

The synthesized bio-adhesive with a different run was analyzed in terms of physical properties like solubility, and acid value.

Determination of acid value (ASTM D664): The acid value of synthesized bio-adhesive was determined according to the ASTM D664 method. 1g of bio-adhesive was dissolved in 20mL of chloroform in a conical flask. Two drops of phenolphthalein indicator were added and titrated up to the formation of pink color with 0.1N potassium hydroxide solution, then the following formula was used to estimate the acid value.

$$Acid\ value = \frac{56.1\ \times V\ \times C}{m} \qquad \text{Eq. 3. 6}$$

Where 56.1 is the molecular weight of potassium hydroxide, V is the volume in mL of standard volumetric KOH solution, C is the concentration of KOH and m is mas of the sample.

Determination of bulk density (ASTM D1076): The density of ETM-*l*-OLLA bio-adhesive was determined according to ASTM D1076 using a metal density cup with a capacity of 100mL. The pre-cleaned density cup was weighed using a digital weighing balance and its weight was recorded as W_1. The ETM-*l*-OLLA bio-adhesive with different mix ratios was filled in the metal density cup and its weight was measured using digital weighing balance and recorded as W_2. Then the density of the ETM-*l*-OLLA bio-adhesive was calculated using the following formula,

$$Bulk\ density = \frac{W2 - W1}{V} \qquad \text{Eq. 3. 7}$$

Solubility test for ETM-*l*-OLLA bio-adhesive: The solubility of the ETM-*l*-OLLA was investigated in a similar procedure to that of the *Euphorbia trigona* Mill latex-based on a method reported by (Bigoniya, Shukla, and Singh 2010). One gram of ETM-*l*-OLLA elastomeric bio-adhesive was dissolved in 10mL of chloroform, acetone, petroleum ether, and water for 12 hours with occasional shaking. Then after 12 hours the residue was separated from the solvent and dried and weight and the solubility.

Surface wettability analysis of ETM-*l*-OLLA bio-adhesive: To investigate the bio-adhesive was washed with distilled water to remove the unreacted and dried in an oven at 105°C until constant weight is maintained. And the adhesive was glued on the glass substrate evenly. Then water was dropped on the evenly distributed bio-adhesive and the image was taken instantly with a high-resolution camera. The image was analyzed with image software to investigate the contact angle of the bio-adhesive and surface wettability.

3.3.2. Functional Group Analysis

The functional groups contained in the synthesized *Euphorbia trigona* Mill latex-lactic acid bio-adhesive and the L-Lactic acid oligomer were determined using Fourier transform spectroscopy analysis. Fourier transform spectrophotometer measured on Spectrum 65 FT-IR (PerkinElmer) in the range 4000-400cm^{-1} at a resolution 4cm^{-1} with four scans using KBr pellets. This experiment was done at Addis Ababa University, college of natural science department of chemistry.

3.3.3. Chemical Structure Analysis

The chemical structure of the ETM-*l*-OLLA bio-adhesive and the OLLA was analyzed with Nuclear Magnetic Resonance spectroscopy. The sample was dissolved in deuterated chloroform ($CDCl_3$) and filtered with a 0.25 μm filter and transferred to a 5mm diameter NMR tube. Similar to the FTIR analysis this experiment was done at Addis Ababa University, college of natural science department of chemistry.

3.3.4. Thermal Property Analysis

The TGA was performed to determine the thermal degradation temperature of the ETM-*l*-OLLA bio-adhesive. Samples were heated from 30°C to 700 °C at the heating rates of 5, 10, 15, and 20 °C/min under nitrogen (N_2) atmosphere.

3.3.5. Antibacterial Analysis

The *in vitro* antibacterial activity of *Euphorbia trigona* Mill latex, ETM-*l*-OLLA bio-adhesive, and OLLA was determined by the disk diffusion method (Kiehlbauch et al. 2000). The medium was prepared by dissolving 38gm of Muller Hinton Agar medium (MHA) in 1000 mL of distilled water and autoclaved at 121 °C for 15 minutes. The autoclaved media was poured into sterile plates (20-25mL/plate) and the plates were allowed to solidify. After solidification, the plate was seeded with an overnight grown culture of approximately 1.5×10^8 CFU/mL by swabbing evenly onto the surface of the medium with a sterile swab.

The samples under investigation that are freshly trapped ETM latex, OLLA, and ETM-*l*-OLLA bio-adhesive at a concentration of 1, 2, 3 mg/mL were prepared by dissolving in DMSO. Whatman filter paper number 1 was used to prepare discs that are approximately 6mm in diameter. The sterile discs were infused with the samples under, and the position on the surface of the medium with sterile forceps and gently pressed down to ensure contact with MHA. Ampicillin was used as a positive control and the plates were inverted and incubated at 37°C for 24 hours. After the incubation, the inhibition zone produced by the three samples was evaluated by measuring the diameter in (mm) of the clear visible zone around the disc against each test sample using sample.

3.3.6. Adhesive Property Investigation of ETM-*l*-OLLA Bio-adhesive

Adhesive nature investigation by drag method: To investigate the adhesive nature of the ETM-*l*-OLLA bio-adhesive towards the peel force experimental setup was done at the laboratory. A commercially available bandage was washed with a solvent to remove the adhesive, and 0.1gm of ETM-*l*-OLLA bio-adhesive was applied to it, and it was glued to a leather substrate. And then the known amount of mass was applied to the glued sample and the time at which the peel started, the mass before the first peel was recorded as the minimum mass, and the mass after the first peel was recorded as the maximum mass. With this method, the adhesive nature of the ETM-*l*-OLLA bio-adhesive towards peel strength was investigated indirectly.

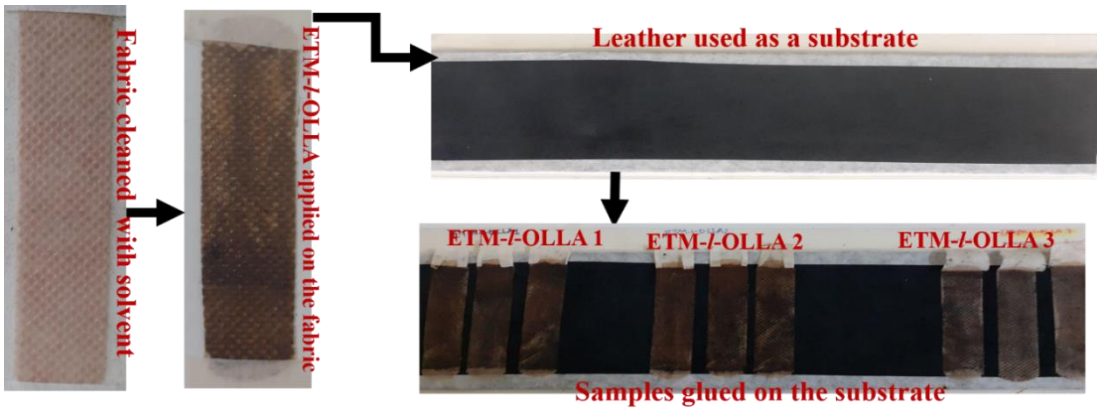


Figure 3. 5: Experimental procedure for adhesive performance test with drag method

CHAPTER FOUR

4. RESULTS AND DISCUSSION

4.1. Physicochemical characterization of *Euphorbia trigona* Mill latex

Determination of physicochemical properties of the given substance helps to know and decide the end application and utilization of that material. These properties include ash content, moisture content, moisture absorption, pH, percentage lipid content, crude protein, carbohydrate content, and functional groups (Salve 2015).

From those physicochemical properties i.e., pH, density, dynamic viscosity, moisture, ash, total resinous matte, and total solid content of freshly trapped *Euphorbia trigona* Mill latex were determined and the result is tabulated in the table (Table 4.1).

Table 4. 1: Summary of results for physicochemical properties of *Euphorbia trigona* Mill latex

Physico-chemical property	**Result**
pH	4.72
Dynamic viscosity	0.062 Cp
Density	1.0496gm/ml
Ash content	5.33%
Moisture content	23.8%
Total solid content	17.65%
Total resinous mater	18.6%

For latex pH value is related to a coagulation process of latex, based on the physicochemical properties that were analyzed. Coagulation occurs when the pH reaches 4.50 - 4.20 and the latex particles have negative electrical charges. Above, the energetic particles repel one another, but not when the pH of the latex is increased to 11 (I, Khan, and Akhtar 2003). The pH value of the ETM latex was found to be 4.72. During the collection of raw material and also during the synthesis process, the coagulation process was one challenge. Different preservation methods are suggested by the researcher but during this research, the latex was stored at low temperature i.e. cooling refrigerator until further use. The moisture, total solid content, weight per milligram (density), ash

content, total resinous matter were found to be 23.8%, 17.65%,1.0496gm/mL, 5.33%, and 18.6% respectively.

The total solid content of latex is related to the viscosity of the latex for this experimental estimation the total solid content is found to be comparable to the literature works from similar plant genus of Euphorbia. The resinous matter is a kind of highly viscous and solid-like substance specifically when it is plant-based it is known to be a mixture of organic compounds. The qualitative estimation of this constituent is found to be comparable by the report of the other research group (Bigoniya, Shukla, and Singh 2010). Regarding the weight per milligrams of the fresh latex, it is found to be higher than water and because of this, the freshly trapped ETM latex is soluble in water.

The other physical characteristic investigated to characterize the *Euphorbia trigona* Mill latex was the solubility test. The capacity of a liquid, solid, or gaseous active ingredient (termed as solute) to disintegrate in a solvent (usually a liquid) and form a solution is referred to as solubility. The liquid (solvent) used, along with temperature and pressure, all have a significant impact on a substance's solubility. Solubility is a physical property of a substance that is related to its nature. In general, the chemical structure influences all chemical and physical properties, including bond type, bond nature, bond angle dimensions of the chemical compound, and the interaction of the molecules in the given compound(Wade 1898). The freshly trapped *Euphorbia trigona* Mill was shade dried; reduced in size using pestle and mortar and dissolved in chloroform, petroleum ether alcohol, and water. The freshly trapped *Euphorbia trigona* Mill latex in its liquid sate was investigated in a similar method for the cured one with the same solvent. Observations for both tests are summarized in table 4.2.

Table 4. 2: Solubility result for *Euphorbia trigona* Mill latex

Solvents	**Fresh latex**	**Cured latex**
Water	Soluble	Insoluble
Acetone	Precipitate	Insoluble
Petroleum ether	Swell	Soluble
Chloroform	Soluble	Soluble

From this investigation, the cured latex was soluble in petroleum ether and chloroform, whereas the liquid latex is completely soluble in water, chloroform, and partially soluble (swells) in petroleum ether. The solubility difference between the liquid latex and the solid (cured latex) can be attributed to the phase change that occurred during the solidification of the latex that is the coagulation of latex. Latex coagulation is generally a chemical process and can be spontaneous or performed in a controlled manner by the addition of chemicals. For this study work, the latex of *Euphorbia trigona* Mill latex undergoes spontaneous coagulation develops due to the interaction of the microorganisms(endophytes) in the latex with non-rubber particles releasing anions which in turn interact with the divalent molecules ions and result in a plug like a polymer that reached an irreversible state (Wahler et al. 2009b). So, from the solubility investigation, it can be confirmed that the constituents in the latex are polar as chloroform is a nearly polar solvent and solubilizes both liquid and cured latex. The solubility variation of the two substances in water is then attributed to the changes that occurred.

4.2. Preliminary Phytochemical Analysis of *Euphorbia trigona* Mill latex

Phytochemicals are defined as biologically active compounds that are produced by plants and vegetables mainly (Mendoza and Silva 2018). The main science that studies those chemicals is known as phytochemistry and its main objective is to study the chemical constituent of the plant including structure, metabolism, natural distribution, biological function, extraction, qualitative and quantitative analysis. Phyto-chemical analysis helps to decide the end-use of the available raw material besides knowing what exactly the plant is. As phytochemicals have different forms and structures it is difficult to give exact classification and so categorized as primary and secondary metabolites. The commonly accepted secondary metabolites are phenols, alkaloids, terpenoids lipids, and carbohydrates (Koche, Shirsat, and Kawale 2016).

Based on the standard testing methods for the phytochemical the freshly trapped *Euphorbia trigona* Mill latex was investigated for the phytochemicals. The test results together with the followed test procedures are summarized in the following table (Table 4.3).

Table 4. 3: Summary of preliminary phytochemical screening investigation

Phytochemical Screening	Test method	Observed
Saponins	Froth forming	Positive
Alkaloids	Mayer's test	Negative
Anthraquione glycosides	Borntrager test	Negative
Tannins	Ferric chloride test	Negative
Phenols	Ferric chloride test	Negative
Flavonoids	Ferric chloride test	Positive
Terpenoids	Salkowski test	Positive
Cardiac glycosides	Keller-killani test	Negative

Notice: Positive indicates presence, Negative indicates absence of the secondary metabolite

From the preliminary phytochemical investigation, three phytochemical components (i.e flavonoids, saponins, and terpenoids) show a positive result. The latex investigated in this study is crude which does not undergo any separation for further utilization of this material the components need to be separated with respective isolation methods. But for now, for this research work isolation was not performed as the modification rather TLC was performed again to see if the components are really available and to decide the next step of the study work.

Thin-layer chromatography: Thin layer chromatography is the kind of chromatographic technique that helps to separate mixtures based on the physical properties of the individual compound and thus its molecular structure, particularly functional groups. As the stationary surface, a thin glass sheet is covered with either aluminum oxide or silica gel is used. The solvent used in the mobile phase is selected depending on the characteristics of the mixture's components (Bele et al. 2011). Here in this research work, the TLC was used to confirm the major component in the freshly trapped *Euphorbia trigona* Mill *l*atex. Various solvent mixtures were used as mobile phase for good resolution and finally, 6DCM:1M was used and one component was visualized for both short (254nm) and long (365nm) lights with a retention factor of 0.75. Having this indication it was hypothesized that the major constituent of the *Euphorbia trigona* Mill latex is one

component as a major constituent where has been confirmed by the NMR analysis which is described in the next portion of this work.

A)  B) 

Figure 4. 1: TLC plate for *Euphorbia trigona* Mill latex with 6M:1DCM A) short B) long wave UV light

4.3. Functional Group Analysis

Fourier transform (FT IR) spectrophotometry is an analytical tool used to determine the chemical constitutes in the organic compound either in solid liquid or gas forms. The technique involves subjection of sample to infrared radiation resulting in atomic vibrations of a molecule in the sample, resulting the giving specific absorption and/or transmission of energy (Nandiyanto, Oktiani, and Ragadhita 2019). Following the step by step analysis procedure the graph have more than five peaks hence this confirms that *Euphorbia trigona* Mill latex is a complex organic molecule.

On the IR spectrum, a broad absorption peak around 3500-3200 cm^{-1} shows intensified availability of the OH group. For the liquid of *Euphorbia trigona* Mill latex absorption peaks, 3595 cm^{-1} shows the water contained in the latex as moisture. The peak at 3357 cm^{-1} can be assigned to OH stretching vibration on the triterpene backbone connected to the (C-3) for the liquid latex. Similarly, this absorption peak was observed at 3323cm^{-1} for the cured latex. Similar absorption peaks for the hydroxyl group on the backbone of terpenes were reported to have nearly similar absorption bands (Shamsabadipour et al. 2013). The peaks at 2962, 2086cm^{-1} show antisymmetric and symmetric stretching of the C-H group. The characteristic peak for $CH=CH_2$ was observed at 1625, 1660cm^{-1} for the liquid and cured latex respectively. The other frequency peak at 1454 and 1374 cm^{-1} (C–O stretch), 1371 cm^{-1} (Q. Q. Wang, Huang, and Wang 2020) phenol or tertiary alcohol (Zafar et al. 2020). In addition the characteristic peaks for both liquid and cured ETM latex at around 1063cm^{-1} shows the C-O-H stretching vibrations.

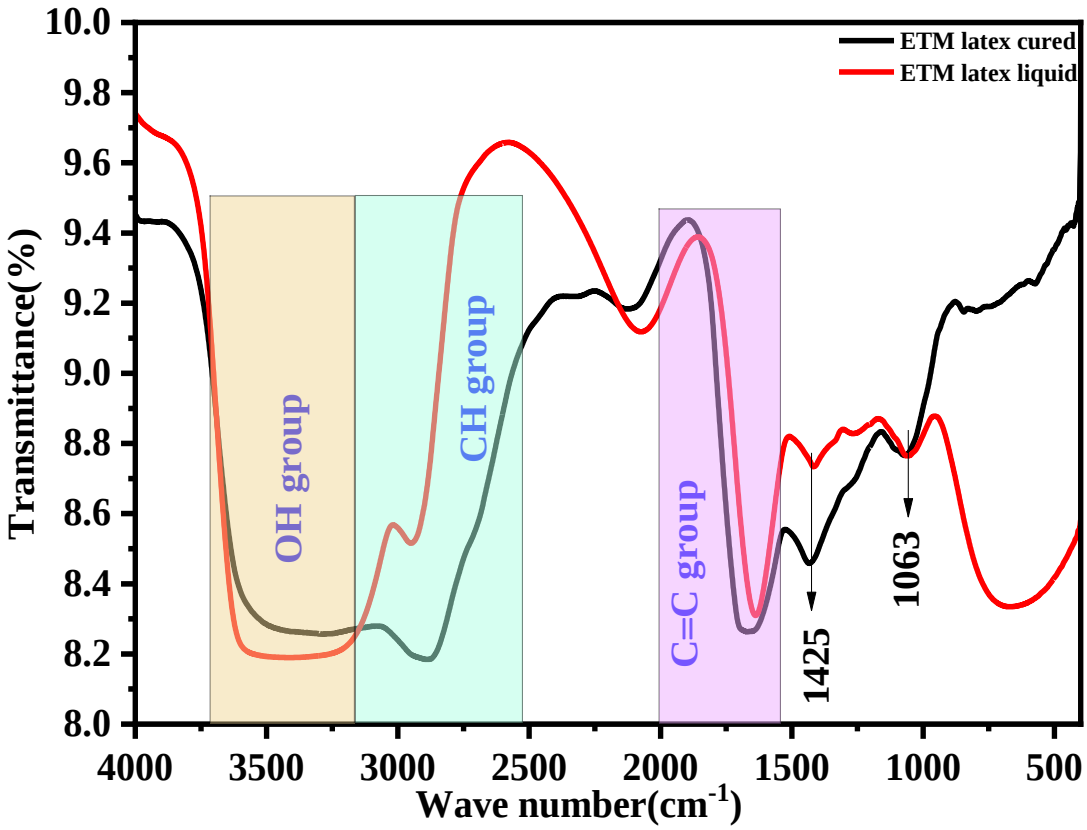


Figure 4. 2: FT IR spectra for liquid latex

As it is shown in the above figure (figure 4.2), the FT IR peak for the cured latex has a difference from that of the liquid latex. The major changes observed are increase and decrease in intensity that is under the single bond area the C-H stretching was found in the wavelength of 2962 cm^{-1} for the liquid latex which appeared at 2762 cm^{-1} reduction in the intensity of the CH_3 and CH_2 that are due to the polymerization process of the latex. The polymerization of latex in other words is knowns as spontaneous coagulation of latex facilitated by the interaction of the unsaturated carbon-hydrogen bonds in the latex. The soluble suspensions in the latex system are trapped by the insoluble part mainly the resin-like organic micro-molecule proceeded by the evaporation of water and the formation of polymer-like material having irreversible nature (Wititsuwannakul et al. 2008). Other than absorption peaks in the single bond area; the formation of new peaks at the fingerprinting of the FT IR at 843cm^{-1} attributed to the ring deformation is additional support for the curing due to spontaneous coagulation. The other characteristic peaks showing the stretching of the C-OH group decreases in intensity for the solid latex supporting the removal of water shown the decrease in intensity of the broad absorption peak around 3595cm^{-1}.

4.4. Chemical Structure Analysis

The chemical structure of a given compound understudy can be investigated with spectroscopy methods like nuclear magnetic resonance (NMR). This analysis is conducted on the principle of, many nuclei have spin, and all nuclei are electrically charged, according to the NMR principle. An energy transfer from the base energy to a higher energy level is achievable when an external magnetic field is supplied (generally a single energy gap). When the spin returns to its base level, and when the spin returns to its base level, energy is emitted at a wavelength that corresponds to radio frequencies that are measured and processed to give an NMR spectrum (Yadav 2005).

For the freshly trapped *Euphorbia trigona* Mill latex NMR spectra were recorded on a Bruker Avance AV 400, using $CDCl_3$ as a solvent and the structure was identified based on (^1H and ^3C NMR) obtained and compared with literature data. Tolerable chemical shift differences were observed while matching the literature data that may arise from different instrumental and procedural differences.

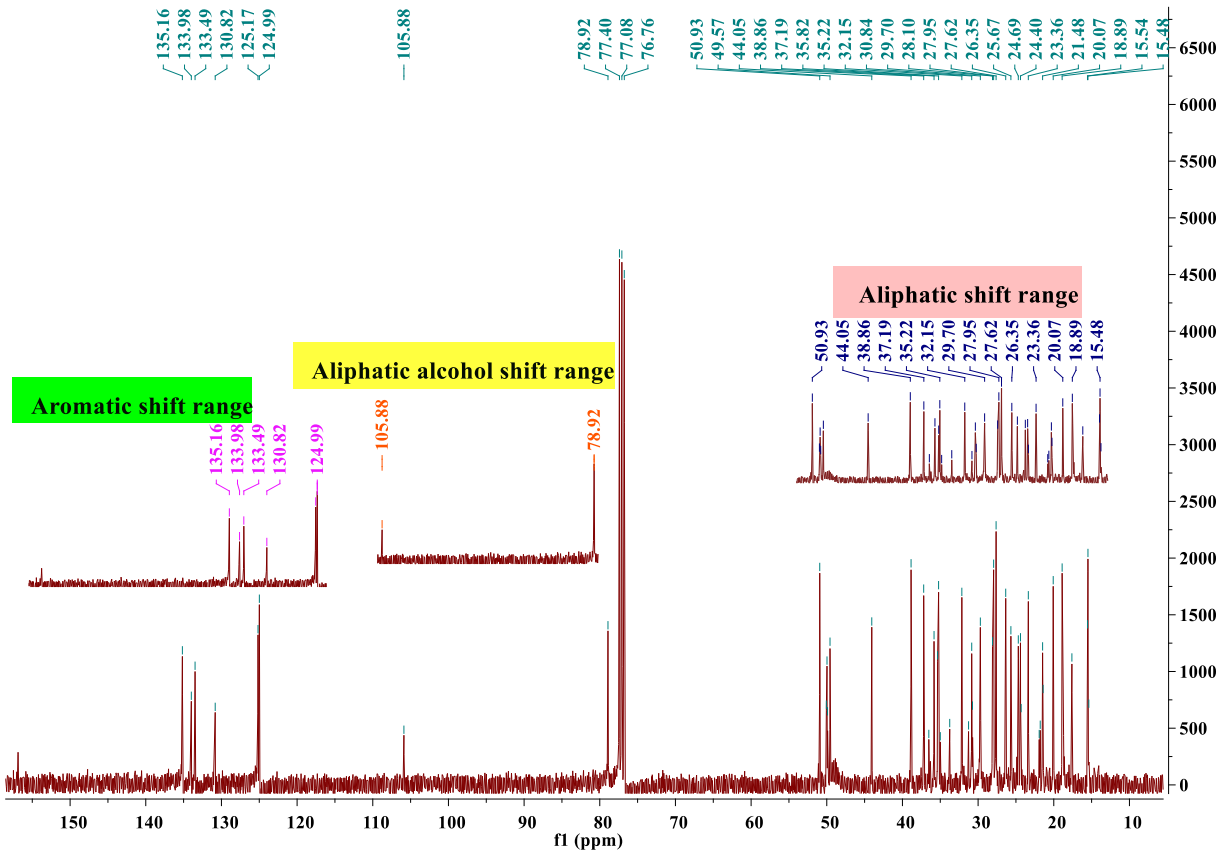


Figure 4. 3: ^{13}C NMR spectrum for freshly trapped ETM latex

^{13}C-NMR: The ^{13}C-NMR spectrum showed 32 carbon signals in the chemical shift between δ C 15.37 ppm to 156.87 ppm. In the aliphatic shift region (0-50ppm), 24 carbons were found. Four spectral signals that show quaternary carbon at (C-8, C-9, C-24, and C-25) were observed for this region with a chemical shift of 49.97ppm, 44.05ppm, 38.86ppm, and 37.19ppm. In the aliphatic alcohol shift region (51-110ppm), two carbon atoms were observed, one signal of a hydroxyl methine carbon at δ C 78.91 ppm (C-3) and the other for the terminal olefin at δ C 105.6 ppm (C-31). In the aliphatic-aromatic shift region (112-160ppm) six carbon atoms were observed at a chemical shift of 124.49ppm, 130.82ppm,133.49ppm, 133.98ppm,135.16ppm, and 156.87ppm (C-11) (Yadav 2005).

^{1}H NMR: The hydrogen NMR shows proton signals in the range of chemical shift between 0.48ppm to 7.36ppm. The hydroxyl proton was observed at chemical shift of δH 3.85 (H-3), olefinic protons were observed at a chemical shift of δH 6.42ppm, δH 5.08ppm-5.01ppm (H-24),

olefinic protons for the methyl(-H_3), methylene(-H_2) and methine (-H) protons were observed at a chemical shift of δH 0.85ppm -1.88ppm.

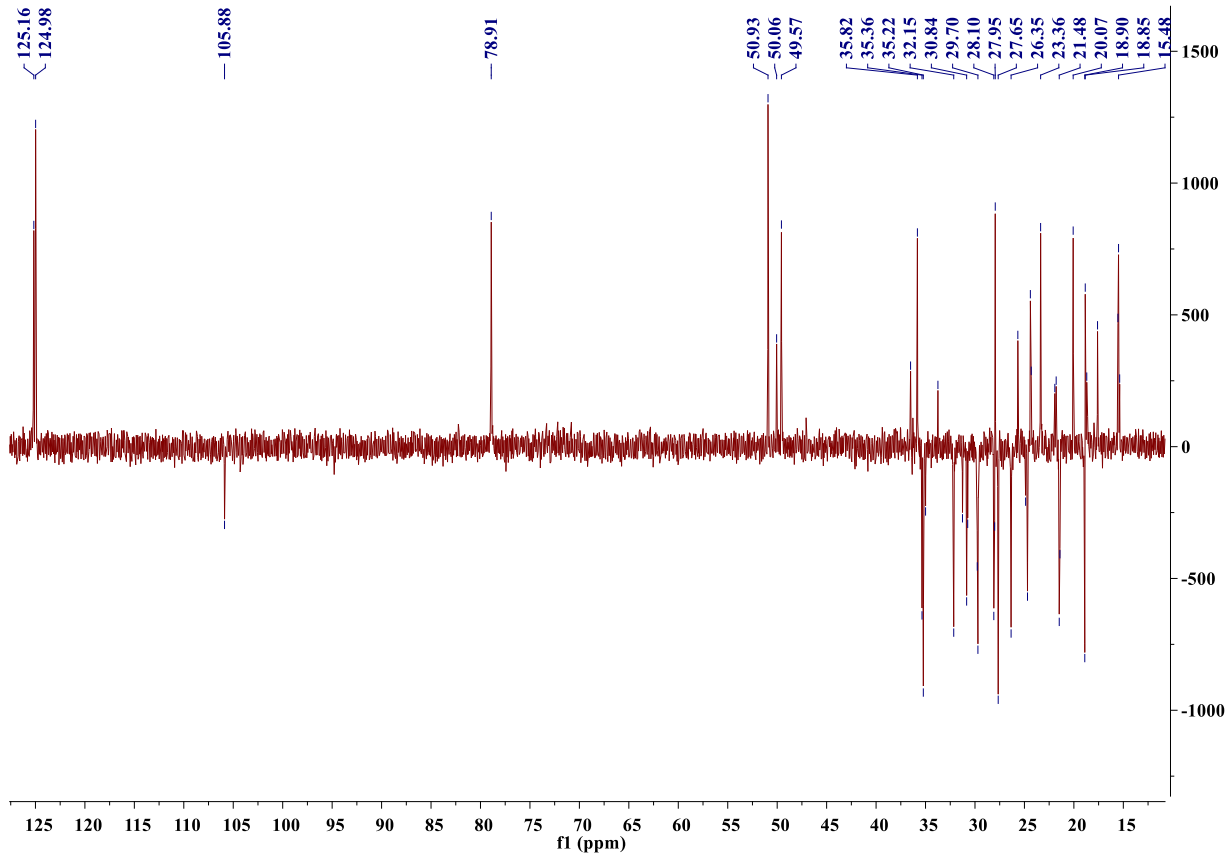


Figure 4. 4: DEPT-135 spectrum for freshly trapped ETM latex

DEPT-135 spectrum: Distortion less Enhancement by Polarization Transfer is the modern ^{13}C NMR spectra that allow to determining the number of attached hydrogens. In DEPT-135 spectrum CH's and CH_3's give positive resonances signals CH_2's give negative resonances signals (Bigler, Melendez, and Furrer 2021). The DEPT-135 spectra were used to identify the quaternary carbon contained in the structure of plant latex as quaternary carbons appear at the ^{13}C NMR spectra and are absent for the DEPT.

Chemical Structure Elucidation: While performing the preliminary phytochemical test it was found that terpene is present in the freshly trapped *Euphorbia Trigona Mill* latex. Based on the observed chemical shift from both carbon and proton NMR and comparing with literature works

(Compounds et al. 2005; Degashu 2017; Duong et al. 2019) we tried to propose the structure of the *Euphorbia trigona* Mill latex.

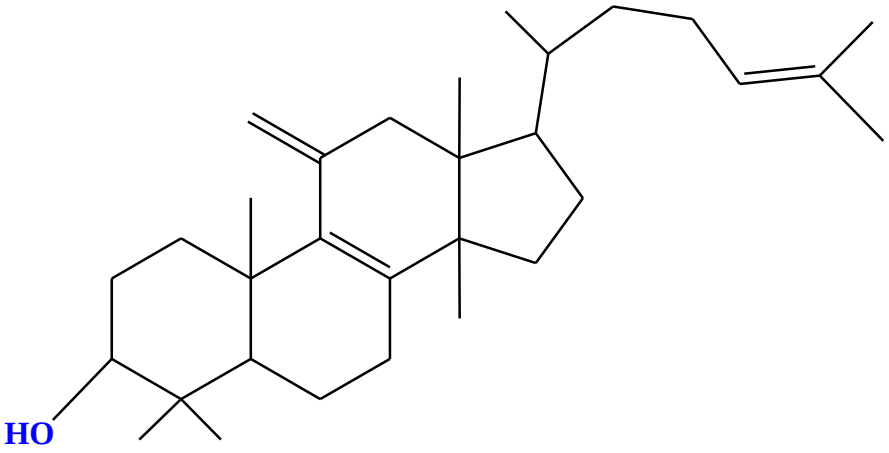


Figure 4. 5: Chemical structure of triterpene from *Euphorbia trigona* Mill latex

This compound was identified as a white crystal and the melting point was investigated with digital melting point apparatus which is found to be 135-137ºC with an uncorrected value. The NMR gives a carbon number of more than 30 with three double bonds this indicates that the structure is a triterpene (Compounds et al. 2005). The spectra for this compound are incomparable in agreement with other triterpene isolated from Euphorbia species (Shamsabadipour et al. 2013). The structure of the triterpene proposed one differs in position of quaternary carbon with a chemical shift of 156.7ppm designated as (C-11). So for a genus of Euphorbia to that of *Euphorbia trigona* Mill species this structure is being reported for the first time up to the time of writing.

Table 4. 4: ^{13}C NMR and ^{1}H NMR for ETM latex

Compound Isolated		**Literature**	**Compound Isolated**		**Literature**
Position (Carbon)	**δ (ppm) ^{13}C NMR**	**δ (ppm) ^{13}C NMR**	**Position (Proton)**	**δ (ppm) ^{1}H NMR**	**δ (ppm) ^{1}H NMR**
1	33.75	34.57	3	3.2(S)	3.23
2	27.62	27.63	18	0.73(S)	0.73
3	78.91	79.20	19	0.97(S)	0.98
4	38.86	39.10	21	0.85(S)	0.86
5	50.93	51.11	24	5.08(S)	5.07
6	18.69	18.92	26	1.58(S)	1.59
7	27.95	27.87	27	1.68(S)	1.66
8	133.89	133.71	28	0.77(S)	0.78
9	135.16	134.22	29	0.97(S)	0.93
10	37.19	37.46	30	0.83(S)	0.83
11	156.87/21.79	21.74			
12	28.1	28.13			
13	44.05	44.30			
14	50.06	50.23			
15	31.26	31.00			
16	29.7	29.93			
17	49.97	49.81			
18	15.54	15.59			
19	20.07	20.35			
20	35.51	35.61			
21	21.41	18.90			
22	35.22	35.45			
23	24.40	24.96			
24	125.17	125.41			
25	130.81	131.01			
26	17.61	17.90			
27	25.69	25.97			
28	24.69	24.69			
29	29.7	28.05			
30	15.48	15.63			
31	105.88				

Cis 1, 4-isoprene unit: The ^{1}H NMR spectra peak at 1.1, 1.7 and 1.23 ppm that appeared as a triplet corresponding to CH_3 proton and the spectra peak at 1.49, 1.58 and 1.66 ppm show the CH where the CH_2 spectra peak is observed at 1.88 ppm. The olefinic proton attached to the carbon to carbon double appears at 5.01, 5.08 and 6.42 ppm. All those ^{1}H NMR spectra chemical shifts suggest that the *Euphorbia trigona* Mill latex contains polyisoprene units.

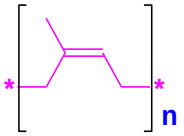


Figure 4. 6: Chemical structure of cis-isoprene unit from *Euphorbia trigona* Mill latex

The ^{13}C NMR spectra shows a characteristic chemical shift of carbon at 23.36 ppm and 26.36 ppm both appearing as a singlet. This carbon is observed at 32.15 ppm. The olefinic carbon peak that is generally expected in the region of 120-140ppm is observed at 124.48, 125.17 ppm corresponding to olefinic ethylene carbon that is $=C-CH_3$. Both the ^{1}H NMR and ^{13}C NMR assigned chemical shifts are incomparable agreement with literature reports and confirm that the polyisoprene unit contained in the latex of ETM latex is cis-1, 4-polyisoprene unit.

Table 4. 5: ^{1}H NMR and ^{13}C NMR for the polyisoprene units in the *Euphorbia trigona* Mill latex

Position (H)	***δ* (ppm) ^{1}H NMR**	**Literature** (Spanò et al. 2012)	**Position (C)**	***δ* (ppm) ^{13}C NMR**	**Literature** (Spanò et al. 2012)
3	1.49	1.46,1.47	1	135.6	135.2
4	5.08	5.15	2	125.17	125.1
5	1.88	1.85,2.25	3	32.15	32.2
6	1.23	1.2,1.29	4	23.36	23.36
			5	26.4	24.69

4.5. Thermal Property Analysis of *Euphorbia trigona* Mill Latex

Thermogravimetric Analysis of Liquid ETM latex: About 10mg of liquid ETM was heated at a heating rate of 20°C/min in a temperature range of 25 to 800°C. The TGA curve for the liquid

ETM latex shows a three-step degradation process. The first degradation step is the dehydration curve in which the water contained as moisture is removed and the low molecular weight volatile components evaporate accounts; for about 65.32% weight loss. This degradation phase appears in a temperature range of 90°C to 217°C with a maximum degradation temperature of 100°C. At its liquid state ETM latex contains a large amount of water and also low molecular weight volatile matters that is why almost more than 50% of its weight loss is observed in the range of dehydration curve. The second weight loss (30.05%) starts in the temperature range of 220°C to 428°C having a maximum degradation temperature of 100°C. This weight loss is due to the degradation of the polymeric material contained in the latex to its monomer units. The final weight for the liquid ETM latex from the TGA curve is found to be 4.06%.

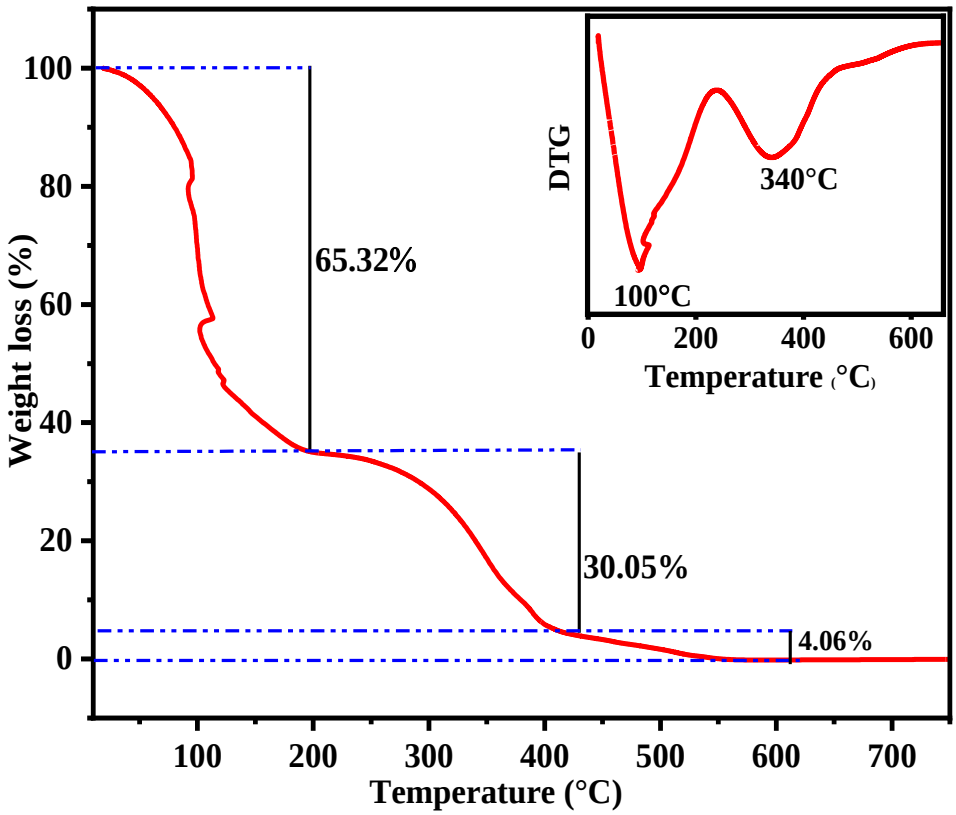


Figure 4. 7: TGA and DG curve for the *Euphorbia trigona* Mill Latex

Thermogravimetric Analysis of Cured ETM latex: *Euphorbia trigona* Mill latex was dried in a shed and the thermogravimetric analysis was performed to study the thermal property of the latex. 10Milligram of ETM latex was heated at a heating rate of 20°C/min in a temperature range of 25 to 800°C and the TGA with DTG curve of the latex exhibited three different degradation

temperatures. The first degradation as weight loss (9.27%) started at an ambient temperature and ends at a temperature of 198°C due to the removal of some amount water and volatile matters contained in the sample. The latex exhibited major weight loss that is 71.89% of its mass at 218-400°C. The final weight loss was observed at the temperature range of 413 to 580°C accounted for 18.77%. From this, the maximum degradation temperature is found to be at 340°C. This experimental data were agreed with the literature data reported for ETM latex by (Anju, Rameshkumar, and Baby 2019). This research team reports the major weight loss for ETM latex at the temperature range of 147-568°C with 76.62% and confirmed the maximum degradation temperature to be 310.47°C.

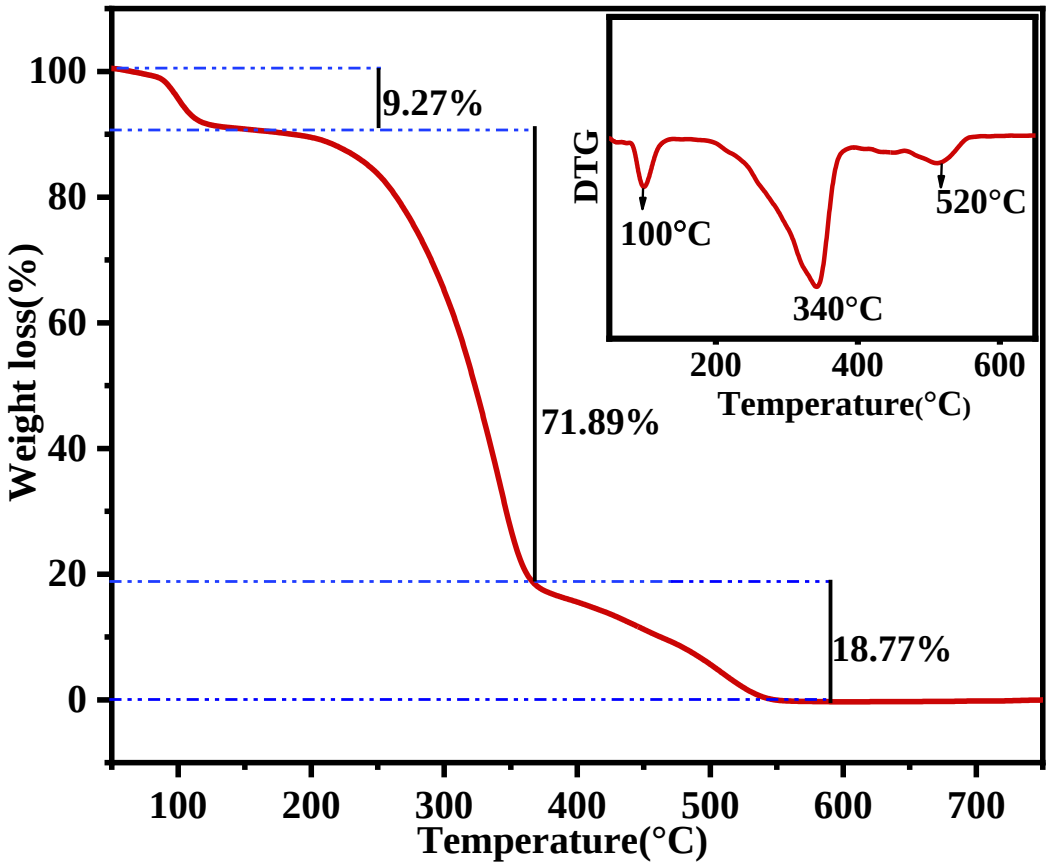


Figure 4. 8: TGA and DTG curve for solid ETM latex

Table 4. 6: TGA analysis of ETM latex

Samples	Temperatures at Different Weight loss (°C)			
	T_{10}	T_{50}	T_{90}	T_{max}
ETM-liquid	83	107	372	100
ETM-solid	188	352	440	340

As tabulated in table 4.6 the liquid ETM and cured ETM latex have different thermal stability. But the TGA curve of the two materials exhibited a similar degradation step that is a three-step degradation process. The difference between those two materials is attributed to the phase change that occurred for the cured latex termed coagulation. As the latex is excluded from the plant and exposed to air it undergoes a coagulation process which gives a polymeric material with irreversible nature. This kind of polymerization is attributed to the polyisoprene units contained in the latex of almost all latex-bearing plants in varying amounts and this material is chemically inert and thus non-toxic (Ramos et al. 2019). When latex coagulates, however, soluble latex components like lipids, waxy-like materials, proteins are trapped in poly-isoprene-based latex micelles forming the water-insoluble polymeric material. The isoprene unit contained in the plant latex is also known as natural rubber and gives elastomeric nature for plant latex thus preserving this natural polymer before it undergoes coagulation by extracting it from the latex with different extraction methods is performed (Azadi et al. 2020; Spanò et al. 2012). For now, under this study, the *insitu* modification was performed with L-lactic acid to synthesize elastomeric bio-adhesive.

4.6. Synthesis and Characterization of *Euphorbia trigona* Mill latex based Bio-adhesive

4.6.1. Reaction Mechanism

ETM latex and lactic acid were used as green starting materials in this study for the synthesis of the bio-adhesive. The hydroxyl group in crude ETM latex is active for reaction, and the carboxylic group from the lactic acid attaches to it via a condensation reaction. L-Lactic acid is esterified, forming an oligomer that attaches to the OH at the backbone of the ETM. The reaction mechanism for the synthesis of ETM-*l*-OLLA bio-adhesive is thought to consist of both lactic acid oligomerizations and the attachment of the OLLA to the backbone of ETM latex. The reaction temperature was set at 150^{o}C because that is the polymerization temperature;

however, experimental reports for lactic acid polymerization in the temperature range of 130^{o}C-180^{o}C are available (Figalla, Petrůj, and Švestková 2018; Miller et al. 2019; Narayanan 2004). The lactic acid (88%) undergoes step growth polycondensation reaction forming ester bonds and releasing water as a byproduct.

In the chemical reaction, temperature is one major factor affecting the complexion of the reaction and the formation of the expected product. Under this study work, the reaction is performed without catalyst, and the temperature is expected to be the main factor to create a collision between the reacting components. The reacting mixture that is the *Euphorbia trigona* Mill latex is a kind of polymeric material. For such material to undergo polycondensation reaction the temperature needs to be higher than the melting temperature (Murillo, Vallejo, and López 2011). On the other hand, if the temperature is below or too much higher there will be no reaction or degradation will happen to the reacting components or to that of the expected product. So to see the effect of the reaction temperature, the carboxylic group titration as an acid value can be seen.

Effect of reaction time on the acid value: The reaction time affects the conversion of reacting components to the desired product. Under this study, the reaction time was investigated starting from 1hour up to 3hours. The reaction is conducted for 1hour, 2hour and 3hour by keeping the reaction temperature and the mix ratio constant. For qualitative determination of the right reaction time the carboxylic acid titration was performed and the acid value at the three reaction times was found to decrease showing the appropriate esterification of the carboxylic groups of the lactic acid. The acid value was calculated from the titration and found to be 246.3, 201.4, and 131.8 for 1 hour, 2 hours, and 3 hours respectively. As it can be seen from the graph below (figure 4.9) the acid value decreased from 246.3 to 201.4 when time changes from one hour to 2 hours again significant change was also observed for the change of reaction time from two hours to that three hours. In addition to this while performing the reaction the amount of byproduct water collected from the react significant change in the amount of byproduct water was observed for a reaction time of 1 and 2, but after 2 hours almost no change was observed in the amount of byproduct water. So this observation again tells as proceeding the reaction more than three hours cannot result in a change in terms of giving the desired product (Tripathi and Katiyar 2018).

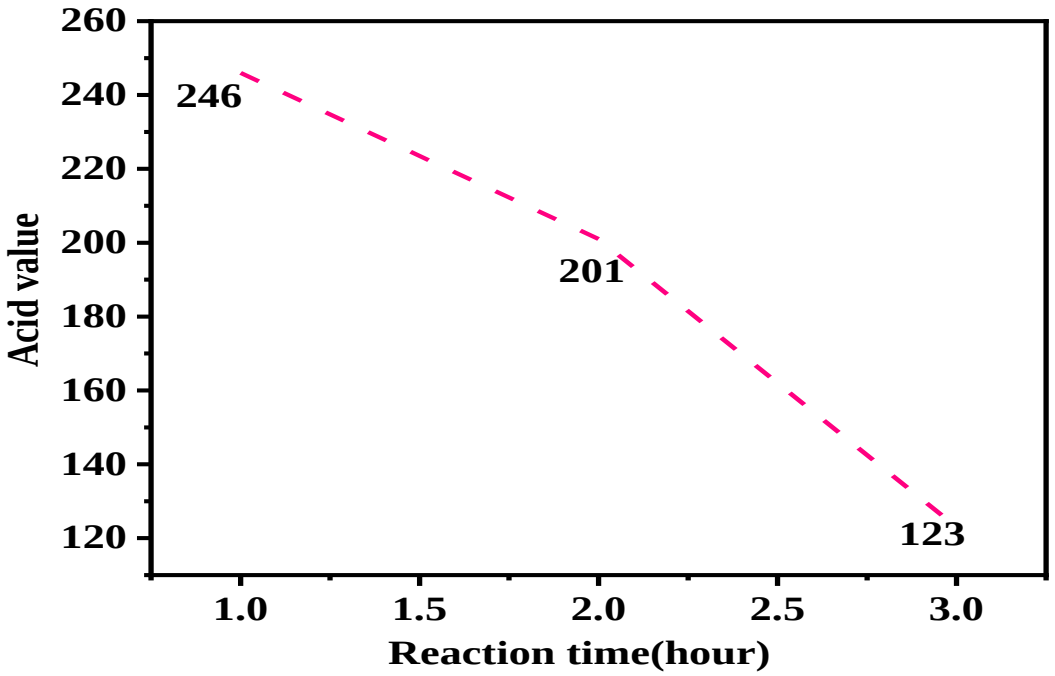


Figure 4. 9: Effect of reaction time on the acid value at150°C temperature and 1:3 mix ratio

Effect of mix on the acid value: The amount of the reacting components that is lactic acid and ETM latex is the other factor to be considered as affecting the completion of the reaction. The amount of lactic acid was kept constant, and the ETM latex was varied, and again the acid value was used to detect the appropriate amount of the reacting component. The acid value was found to be 185.13, 140, and 131.835 for 1:1, 1:2, and 1:3 respectively. A slight change was observed for the change in the amount of the ETM latex from 1 to 2 to 1:3 when compared to the acid value of the 1:1.

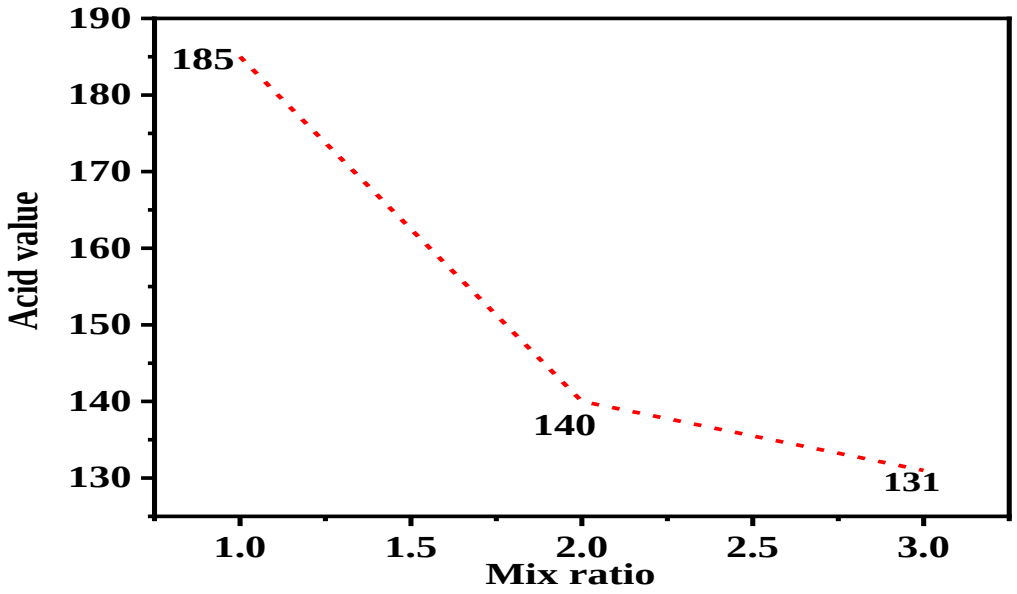


Figure 4. 10: Effect of mix ratio on the acid value at 150°C temperature and 3hours

Effect of temperature on acid value: In the chemical reaction temperature is one major factor affecting the complexion of the reaction and the formation of the expected product. Under this study work, the reaction is performed without catalyst and the temperature is expected to be the main factor to create a collision between the reacting components. The reacting mixture that is the *Euphorbia trigona* Mill latex is a kind of polymeric material. For such material to undergo polycondensation reaction the temperature needs to be higher than the melting temperature (Bell et al. 2019). On the other hand, if the temperature is below or too much higher there will be no reaction or degradation will happen to the reacting components or to that of the expected product. So to see the effect of the reaction temperature the carboxylic group titration as an acid value can be seen. The acid value decreased from 199 to 131 as the reaction temperature changed from 130°C to 150°C, where a slight change was observed for increased reaction temperature from 150°C to 170°C, this decrement can be attributed the reverse reaction of the reaction of the lactic acid oligomer at higher temperature (Bakare et al. 2014) which is non favorable for this process.

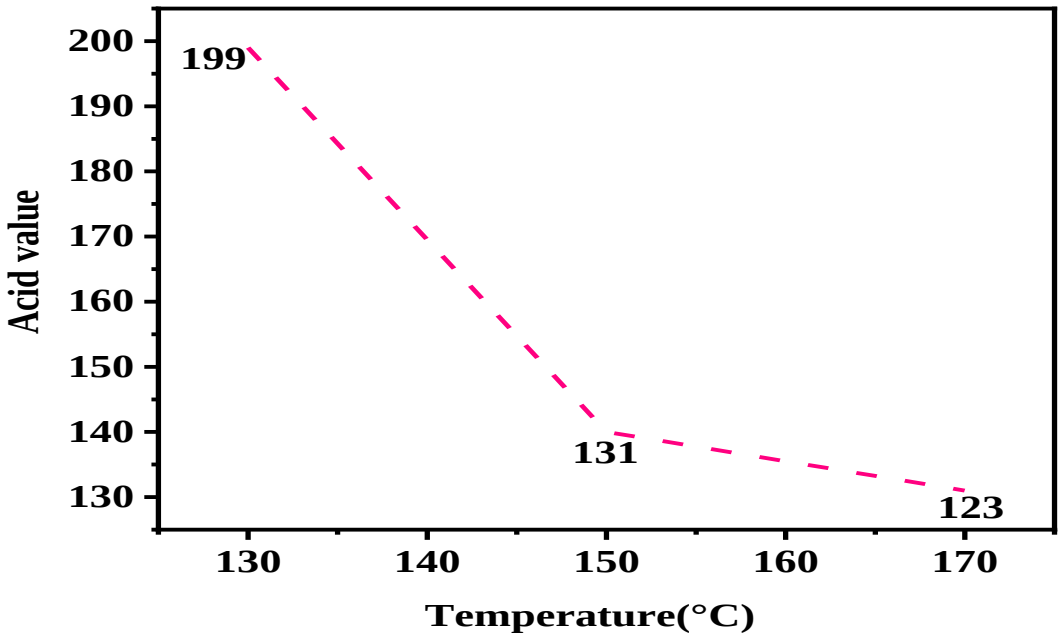
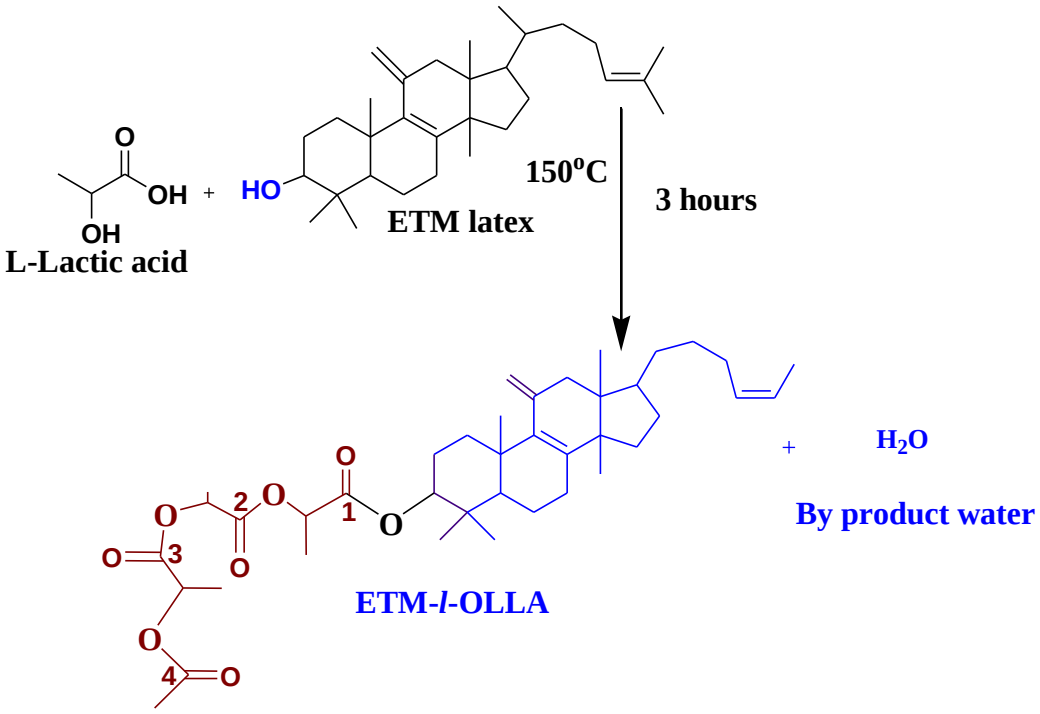


Figure 4. 11: Reaction temperature on acid value at 3hours with mix ratio of 1:3

The ideal reaction mechanism showing the attachment of the OLLA on the backbone of ETM latex can be presented in the following scheme (scheme 4.1).



Scheme 4. 1: Schematic representation of reaction mechanism for the synthesis of bio-adhesive

4.6.2. Physical Tests for the Synthesized ETM-*l*-OLLA Bio-adhesive

After synthesize of the bio-adhesive to insight for instrumental analysis some physical tests like bulk density and solubility were performed.

Bulk density determination of ETM-*l*-OLLA bio-adhesive: The bulk density of ETM-*l*-OLLA conjugate was determined based on ASTM D1076 using a metal density cup for different mix ratios at room temperature. The bulk density of a material is one of the physical properties of a substance can be used to investigate the effect of the synthesized parameters that is the mix ratio. The result for the three ETM-*l*-OLLA adhesive has summarized in the following table (Table 4.7). The increase in bulk density is believed to be the result of an increase in molecular weight. As the lactic acid oligomer takes part and attaches to the ETM latex backbone simultaneously then the molecular weight of the resulting polymeric matter is expected to increase in general(Carbone and Lue 2010) hence resulting in the increased bulk density.

Table 4. 7: Bulk density determination of ETM-l-OLLA bio-adhesive

Mix ration	Product	Bulk density(g/mL)
1:1(LA:ETM latex)	ETM-*l*-OLLA 1	1.0229
1:2(LA:ETM latex)	ETM-*l*-OLLA 2	1.1258
1:3(LA:ETM latex)	ETM-*l*-OLLA 3	1.157

Solubility study of ETM-*l*-OLLA bio-adhesive: to see the change regarding the solubility nature of the synthesized bio-adhesive with respect to the mix ratio the solubility of the synthesized bio-adhesives that is ETM-*l*-OLLA 1, 2, and 3 were investigated in water and other three organic solvents in a similar procedure to that of the ETM latex. All the three adhesives were completely soluble in chloroform, and swell in petroleum ether, on the other hand, those products were found to be swell in water. Solubility is the other physical property of an organic material that can be used to estimate the structural nature of the given material and investigate the change that occurred during the modification undergone by the chemical reaction. On this basis, the change in the solubility of the ETM-*l*-OLLA is the result of the change inappropriate consumption of the hydrophilic groups in the system (Tripathi and Katiyar 2018).

Water Contact Angle Analysis: The angle formed by a droplet's surface and the surface it is dropped on is regarded to as the water contact angle. It is used to qualitatively describe surface wettability and is related to the adhesion nature of the surface. When the water contact angle is small, adhesive forces overweigh cohesive forces, and molecules interact with solid molecules instead of liquid molecules. Cohesive forces are bigger and more powerful than adhesive forces when the contact angle is large, and liquid molecules interact with each other more than solid molecules (Huhtamäki et al. 2018). The water contact measurement was performed in this study work by dropping distilled water on the synthesized ETM-*l*-OLLA bio-adhesive poured on a substrate, which is referred to as a surface in this case. A high-resolution camera was used, and the photograph was managed to capture and analyzed accordingly using Image*J* software (figure 4.12).

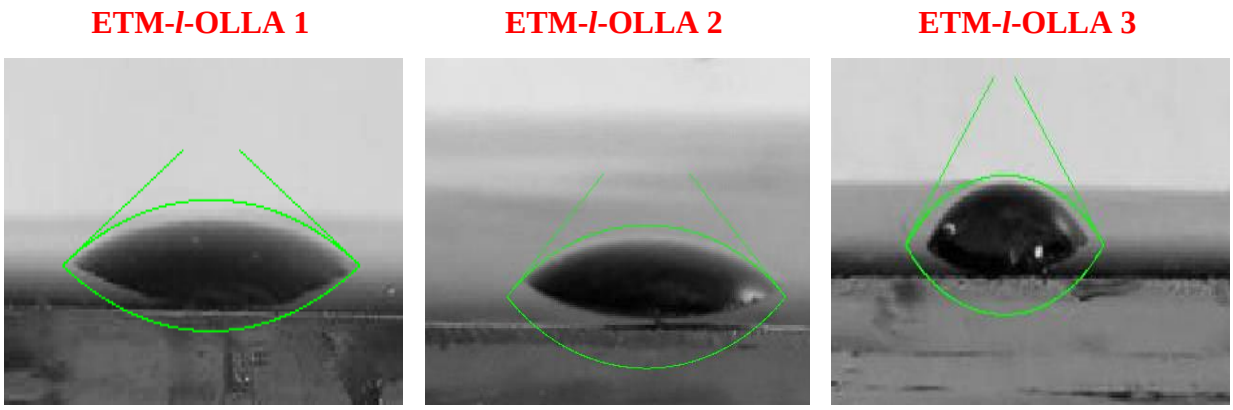


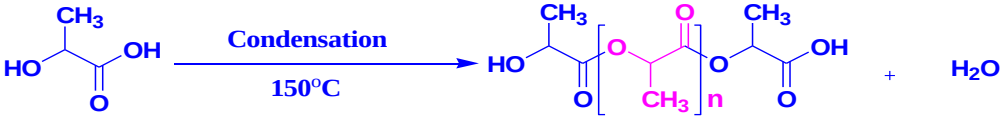
Figure 4. 12: Images for water contact angle of ETM-*l*-OLLA from Image*J* software

The measured water contact angles for the three ETM-*l*-OLLA bio-adhesive matrice, ETM-*l*-OLLA 1, 2, and 3, were 30.78, 47.41, and 53.83, respectively. According to this analysis, the amount of ETM latex increases the change in water contact angle. The increment in contact angle as the mix ratio increase shows the possible replacement of the polar hydrophilic groups available on the system by the newly formed ester groups of the OLLA (Tripathi and Katiyar 2018). Highly increased contact angle that is superhydrophobic surface indicates low surface energy and low adhesion surface where the super hydrophilicity can also result in strong adhesion (Stanton et al. 2012). Thus for the synthesized ETM-*l*-*OLLA* bio-adhesives, the maximum hydrophilic nature is obtained at an increased mix ratio that is for ETM-*l*-OLLA 3 which can be taken as enough as it

can be applicable for a medical adhesive bandage. Or the hydrophobicity can be increased by increasing the amount of the ETM latex.

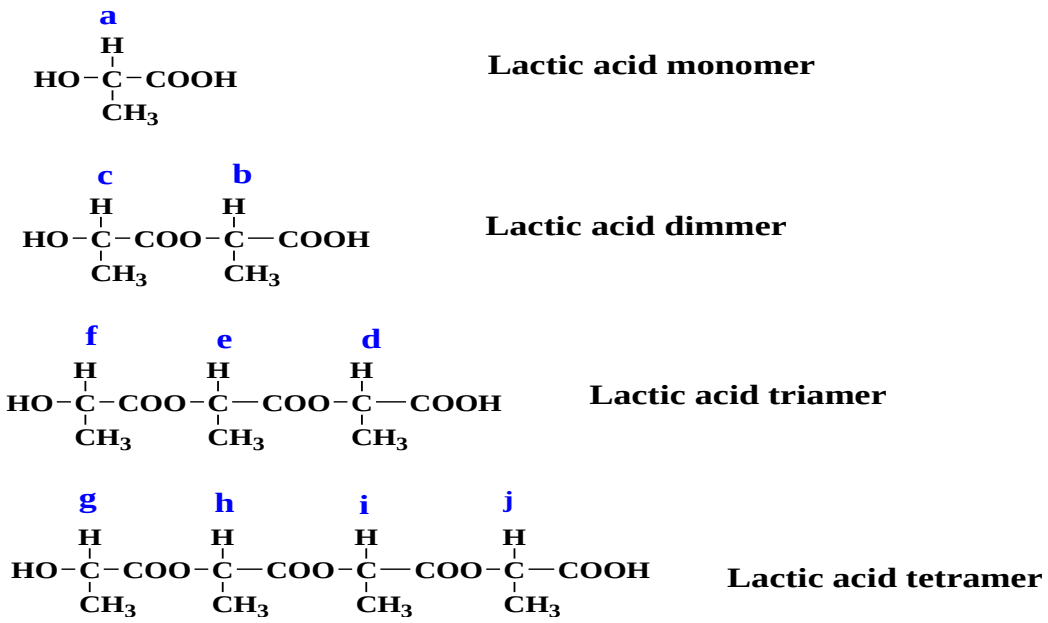
4.6.3. Chemical Structure Analysis

L-Lactic acid oligomer: to investigate and confirm the possible modification that appeared during the synthesis of *Euphorbia trigona* Mill latex lactic acid bio-adhesive defining the chemical structure of the starting material is necessary. As it is explained in the L-lactic acid (88% purity) was expected to undergo step-growth polycondensation reaction and then attach to the backbone of ETM latex. To confirm this L-Lactic acid (88%) was let to be oligomerized at 150ºC and three hours with a similar experimental procedure to that of bio-adhesive and ^{1}H NMR and ^{13}C NMR analysis was performed. The reaction mechanism followed for the step-growth polycondensation reaction of lactic acid can be presented as shown in scheme 4.2 which is the known method.



Scheme 4. 2: Step growth polycondensation of lactic acid polymer

The result of step-growth polycondensation of lactic acid is expected to have repeating units of its starting moiety up to the trimer and tetramer. The possible consecutive repeating units resulted can be presented as in scheme 4.4 and the NMR data can be used and compared to the earlier literature reports to assign and describe the units (Espartero et al. 1996). From the scheme presented below the possible positions for the methine and methane protons expected in the lactic acid oligomer of this work is in the lactic acid monomer as free starting units, at the first position next to the OH esterified group (for units of c, f, and g) at the terminal near to the –COOH end group (for units of b, d, and j) and in the middle in between the two –COO- groups (for units of e, h, and i). So the two basic units in this oligomer CH and CH_3 can be distinguished as OH free and OH esterified groups this information helps to assign the chemical shift as their position results in the difference because of chemical shift is linearly related to electronegativity (Singh, Anthony, and Chowdhury 2018).



Scheme 4. 3: Schematic representation of repeating units of lactic acid oligomers

From the ^{1}H NMR spectrum, the chemical shift for the methine and protons in free lactic acid is found to be 4.03ppm and the methane proton has spectra at 1.23ppm with high intensity. For the consecutive steps, those units result in less intensity this is used to assign their peaks; as the chemical shift is linearly related to the electronegativity. For the methine, and methane groups attached to the OH esterified group deshielding of the first hydrogen is expected than that of the hydrogen at the second resulting in the chemical shift of 4.93ppm and 1.23ppm with downfield resonance signals in the b unit of the dimmer, where the methane methine groups attached to the –COO- group appear as up filed resonance signals with a chemical shift of 4.36ppm and 1.43ppm. This unit of LA oligomer revealed a chemical shift of 4.28, 4.36, 5.1ppm for CH and 1.43, 1.40, 1.55pmm for the CH_3. The last positions of those group for this work is in tetramer of the OLLA with a chemical shift of 4.30ppm, 4.93, 5.12, and 5.16 for the CH and 1.32, 1.40, 1.45, and 1.55ppm for the CH_3 all those possible assignments are summarized in the table below for more simplicity (table 4.8).

Table 4. 8: ^{1}H NMR chemicals shifts (δ, in ppm) hydrogen types in the lactic acid oligomer

	Monomer	**Dimer**		**Trimer**			**Tetramer**			
	A	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**
δ (CH)	4.03	4.93	4.28	4.36	5.10	4.30	4.93	5.16	5.12	4.30
δ (CH_3)	1.23	1.38	1.43	1.40	1.55	1.44	1.40	1.45	1.55	132

In a similar way to that of the ^{1}H NMR, the ^{13}C NMR of lactic acid can be used to describe the carbon type in the consecutive units of CH, $CH_{3,}$ and CO. The assignments for the CH, $CH_{3,}$ and CO units can be done by comparing the ^{13}C NMR spectrum of the synthesized lactic acid oligomer to the literature data (Espartero et al. 1996) in similar to that of the ^{1}H NMR and scheme 1. The chemical shift from the ^{13}C NMR spectrum reveals the carbon types based on the atom they are attached to (Jarmelo et al. 2012). On this basis, the lactic acid oligomer in this work contains three types of carbons that are the carbonyl carbon attached to the oxygen atom, the methine carbon attached to one hydrogen atom, and the methyl carbon, the carbon atom attached to the three hydrogen atoms. The carbonyl carbon of this oligomer can have a possible position at the first of the chain nearly next to the OH attached carbon with a chemical shift 174.9ppm for the dimer, 173.95ppm, and 169.67pp for the trimer and tetramer respectively. The other position of this carbon is in the middle of the chain in between carbons of e and d units (scheme 3) having a chemical shift of 169.9ppm for the tetramer and in between h and i, i and j units (Scheme 3). The last possible position for this carbon type is at the end chain as a COOH group with a chemical shift of 178.67ppm for the free lactic acid monomer, 169.67ppm, 174.9ppm for the dimer triamer and tetramer respectively. Similar to that of the hydrogen types the carbon types assignment can be done for the other two carbons that is the methine carbon and methane carbons and summarized in table below (table 4.9).

Table 4. 9: ^{13}C NMR chemical shifts for the CO, CH, and CH_3 in the lactic acid oligomer

	Monomer		Dimer		Triamer			Tetramer		
	A	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**
δ(CO)	178.67	169.67	174.90	169.67	169.9	173.95	169.67	169.9	169.9	174.9
δ(CH)	66.49	66.72	66.49	69.67	69.06	66.49	69.00	69.03	66.49	66.49
δ(CH_3)	20.02	16.63	20.12	16.56	16.50	19.89	16.56	16.50	16.50	19.89

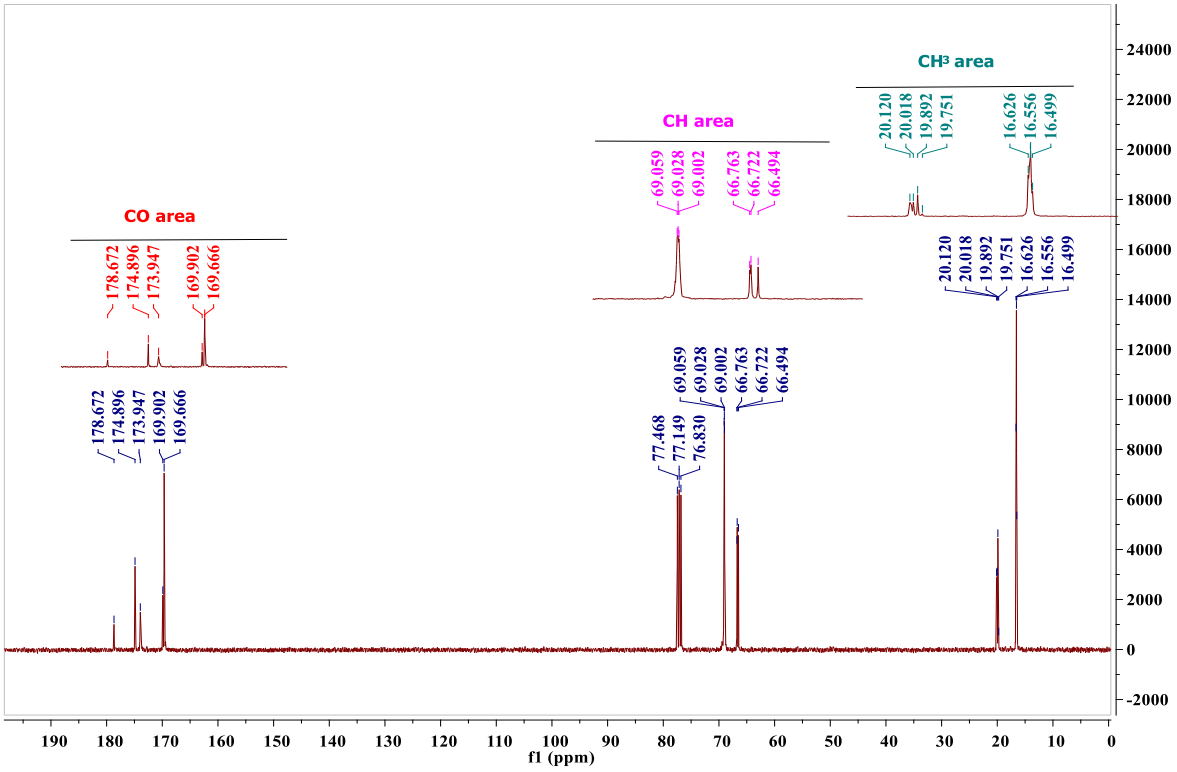


Figure 4. 13:^{13}C NMR of lactic acid oligomer

So from both ^{1}H NMR and ^{13}C NMR the lactic acid that is developed on the backbone of *Euphorbia trigona* Mill latex with a homopolymer having oligomer up to tetramer. This information is used for the interpretation of the synthesized ETM-*l*-OLLA bio-adhesive in comparison with the NMR spectrum of the *Euphorbia trigona* Mill latex.

ETM latex-lactic acid bio-adhesive: Those experimental data from the literature were used for a comparison to decide the oligomerization of the lactic acid on the backbone of ETM latex. As it is expected in this synthesis method the lactic acid monomer forms an oligomer by reacting with the end groups of the OH and the COOH ends of it and then it attaches to the backbone *of Euphorbia trigona* Mill latex through the OH group of the latex.

The lactic acid monomer is expected to undergo oligomerization after the attachment of the OH backbone of the latex. This is confirmed by the NMR analysis of the synthesized bio-adhesive. The ^{1}H of the synthesized bio-adhesive shows spectral shifts at 1.20-1.55 ppm attributed to the protons of the lactic acid oligomer and the methine proton spectral chemical shift at 5.02-5.19 ppm (Zahir et al. 2021). The appearance of those proton peaks confirms that the oligomer of lactic acid has been formed.

Furthermore the ^{13}C NMR shows peaks for that are attributed to the ester carbons from the lactic acid oligomer. The carbonyl carbon at δ 178.60ppm, δ 175.69pmm, δ 175.00ppm, δ 173.73ppm, 173.56ppm shows the attached lactic acid oligomer on the ETM back bone at the OH active site.

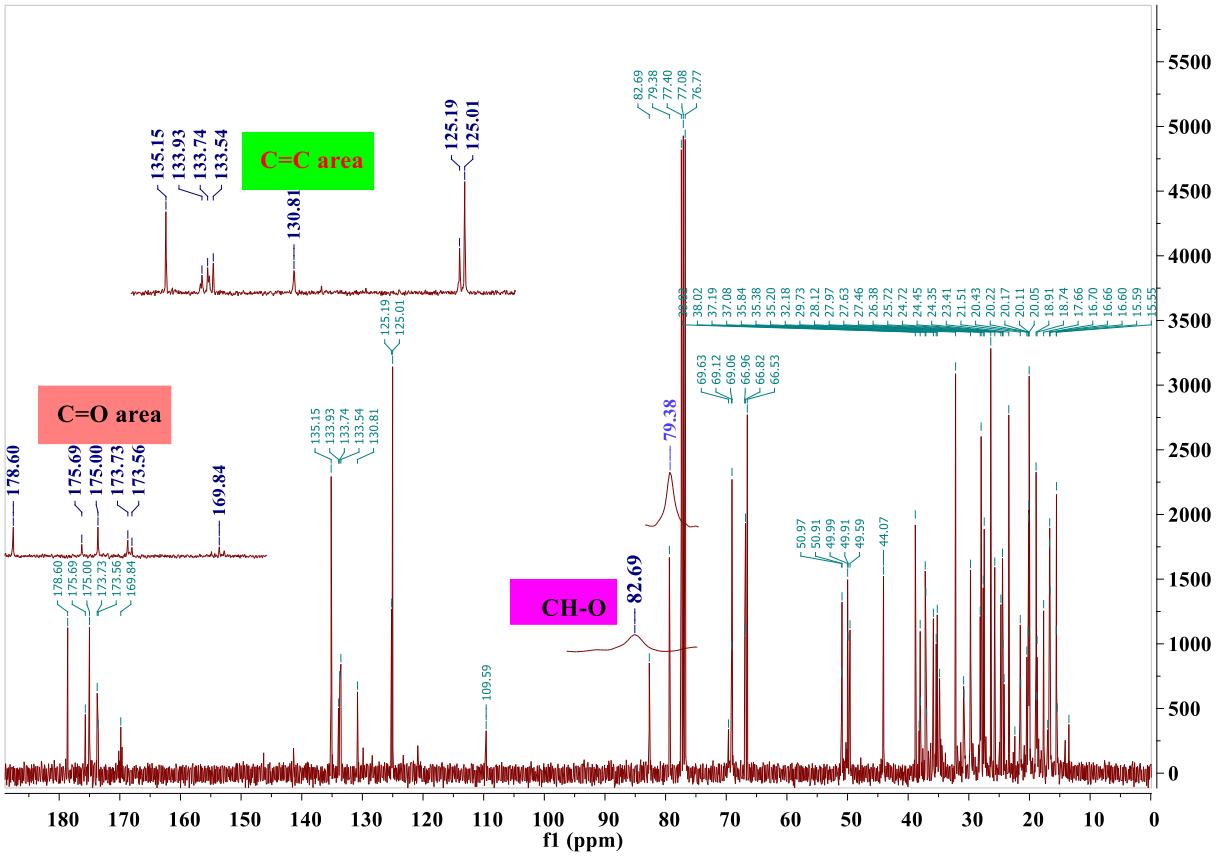


Figure 4. 14: ^{13}CNMR spectrum of synthesized bio-adhesive

The attachment of the L-Lactic acid oligomer to the *Euphorbia trigona* Mill latex backbone took place at the hydroxyl group. This is confirmed by the NMR analysis for the synthesized bio-adhesive. From the ^{13}C-NMR of the bio-adhesive, the carbon (C-3) atom attached to the OH group has shown a change in the chemical shift from 78.91 ppm to 79.38ppm followed by the formation of a new peak at 82.69 ppm. The carbon atom near to the hydroxyl attached carbon (C-2) δ= (27.97ppm), (C-4) δ= (38.8ppm) and also all carbon atoms on the ring have shown a change in chemical shift. (C-1) δ= (34.83ppm), (C-2) δ= (27.97ppm), (C-4) δ= (38.02 ppm), (C-10) δ= (37.9 ppm). The quaternary carbon atom having 156ppm shifted to higher due to the deshielding effect that resulted from the attachment of the OLLA to the ETM latex. Other than this carbon the terminal olefinic carbon at 105.88ppm shifted to 109.9ppm confirming the hypothesized attachment. In general, this change in chemical shift is believed to result from the space interaction between the molecules in the system through a positive inductive effect (Baran et al. 2018).

Other than the main reaction during the attachment of the lactic acid oligomer to the backbone of the latex, addition reactions of the unsaturated double bond that are available at the backbone of ETM latex take place, and consecutive single bonds are generated in the aliphatic region (0-50ppm).

Functional group analysis of the bio-adhesive: The functional group of the synthesized bio-adhesive was analyzed by Fourier transform spectroscopy with comparison to the *Euphorbia trigona* Mill latex and lactic acid oligomer. The changes observed from both materials were used as additional evidence for the modification process. The broad absorption peak observed at 3419cm^{-1} shows the OH group that is attributed to the OH phenols of the ETM latex left unreacted or the OH terminal of the lactic acid (Jarmelo et al. 2012). When compared to the starting materials that are ETM latex and OLLA this peak shows the availability of the hydroxyl group has become narrowed and decreased in intensity, this shows the decreasing of the OH groups in the system as the water is being removed. The characteristic peak observed at the product i.e 2979cm^{-1} attributed to CH_3 asymmetric stretching results from shifts from both lactic acid and latex followed by the formation of a new peak at 2912cm^{-1} assigned as CH_3 symmetric stretching this confirms the possible interaction between unsaturated polyisoprene units. The spectral peak observed at 1739cm^{-1} was assigned as stretching of the C=O group. A similar absorption peak was reported with similar assignment for modification of natural rubber latex with hydrophilic grafting monomer (Kangwansupamonkon, Gilbert, and Kiatkamjornwong 2005).

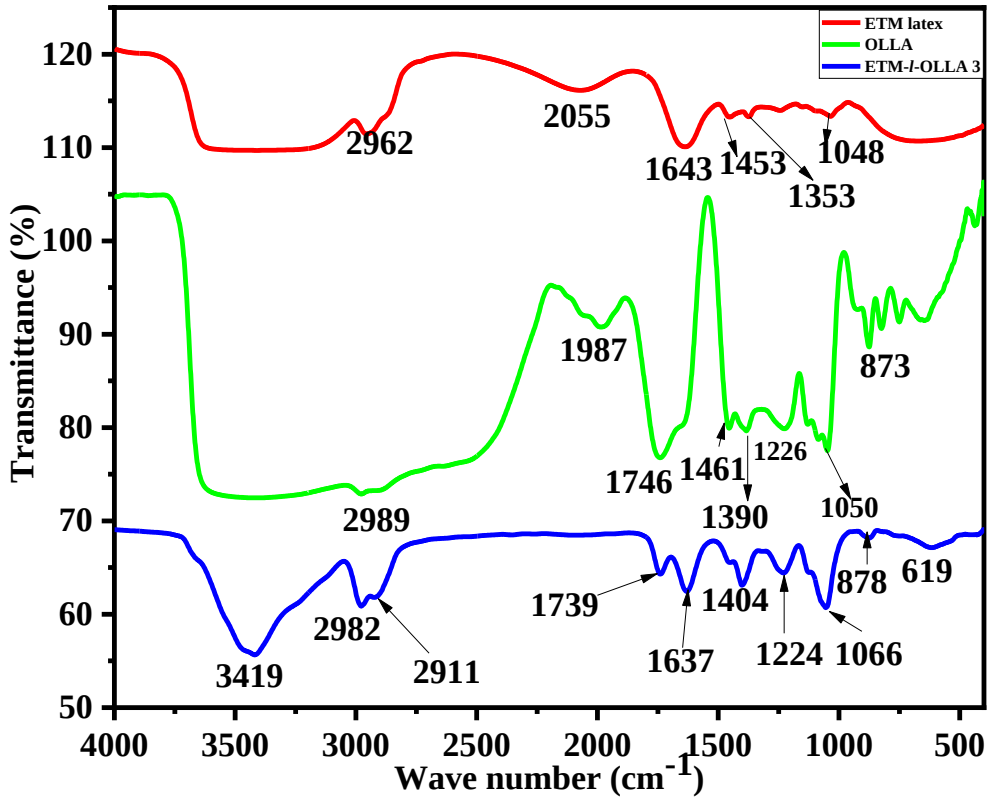


Figure 4. 15: FT IR graph for the liquid latex, OLLA, and ETM-*l*-OLLA-3 bio-adhesive

The IR spectrum can also be used to compare the change that occurred by the change in the mix ratio of the starting material. Figure 4.16 shows the FT IR spectrum for the ETM-*l*-OLLA, ETM-*l*-OLLA 2, and ETM-*l*-OLLA 3 bio-adhesive. The absorption band at 3400 to 3212 cm^{-1} for the three adhesive matrix shows the change in shape in which becomes narrower for the ETM-*l*-OLLA 3 showing that the inter and intramolecular interaction has taken place possible and water has been removed. This changed absorption band for the ETM-*l*-OLLA 3 shows phenolic OH attributed to the triterpene OH (Q. Q. Wang, Huang, and Wang 2020). The specific absorption band at 1700 cm^{-1} (region A) showing the C=O of the lactic acid has decreased where the absorption band for the ETM latex C=C (region B) increased in turn. Moreover, the characteristics absorption bands for the CH bending's (region C) of the terminals of lactic acid at 1448 cm^{-1} for ETM-*l*-OLLA 1 and ETM-*l*-OLLA 2 have disappeared where the absorption band with an overlapping pattern at 1373 cm^{-1} increased in intensity for ETM-*l*-OLLA 3. This phenomenon shows the possible interaction of lactic acid CH terminals with the unsaturated CH of the ETM latex (Pawlak and Mucha 2003). In addition to this, region D of figure 4.16 shows characteristic peaks for the C-O

stretching, and symmetric C-C stretching is seen. The absorption bands for the C-O- stretching showing the cyclic ethers have been changed to more intense and strong bands for ETM-*l*-OLLA 3 than the smaller bands of the ETM-*l*-OLLA1 and ETM-*l*-OLLA 2.

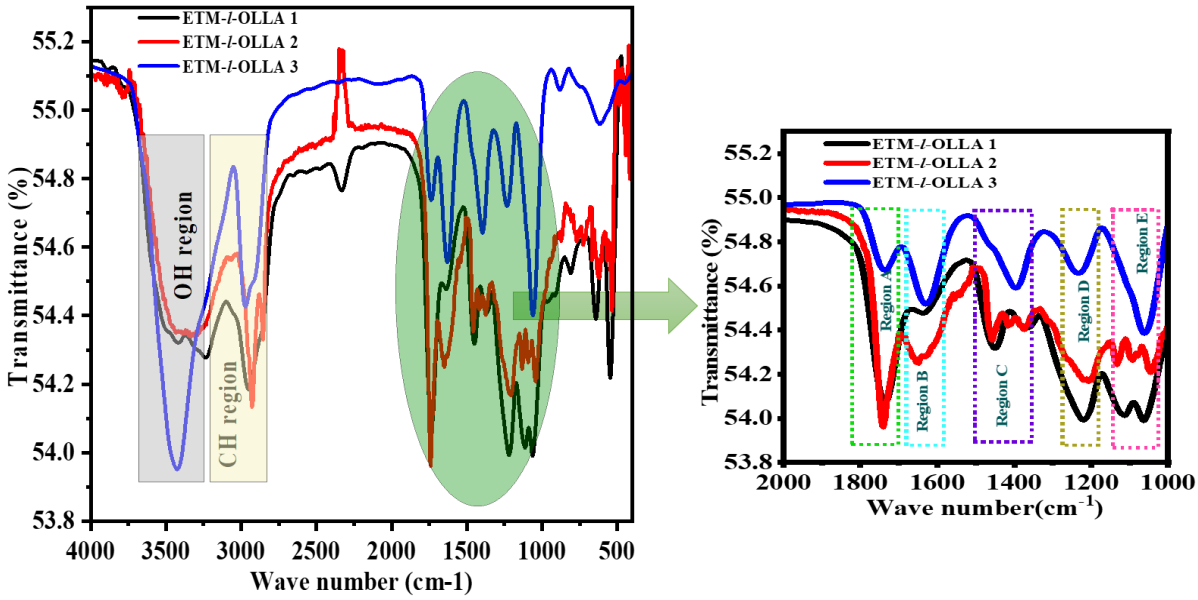


Figure 4. 16: FT IR spectrum for ETM-*l*-OLLA 1, ETM-*l*-OLLA 2, and ETM-*l*-OLLA 3 bio-adhesives

4.6.4. Thermal Property Analysis:

The thermal property of the ETM-*l*-OLLA-3 bio-adhesive was investigated with the thermos-gravimetric analyzer under the heating rate of 20°C/min at a temperature range of 25 to 800°C. The TGA curve was generated by plotting the weight loss versus temperature and it was found to exhibit a two-step degradation process. As it can be seen from TGA and DTG curve (figure 4.17) the first degradation takes place in the temperature range of 120°C to 627°C. The weight loss in this region is found to be 82.02%. The second degradation starts at 399°C and ends at 627°C with a weight loss of 17.98°C and this bio-adhesive exhibited major weight loss of 82.02% with a maximum degradation temperature of 370°C. The first degradation step shows either the evaporation of low molecular weight molecules from the EMT-*l*-OLLA 3 or the chain scission and cross-linking as the ETM latex contains high molecular weight polymeric material which does not undergo major degradation in the temperature of 200-270°C (Rao and Johns 2008).

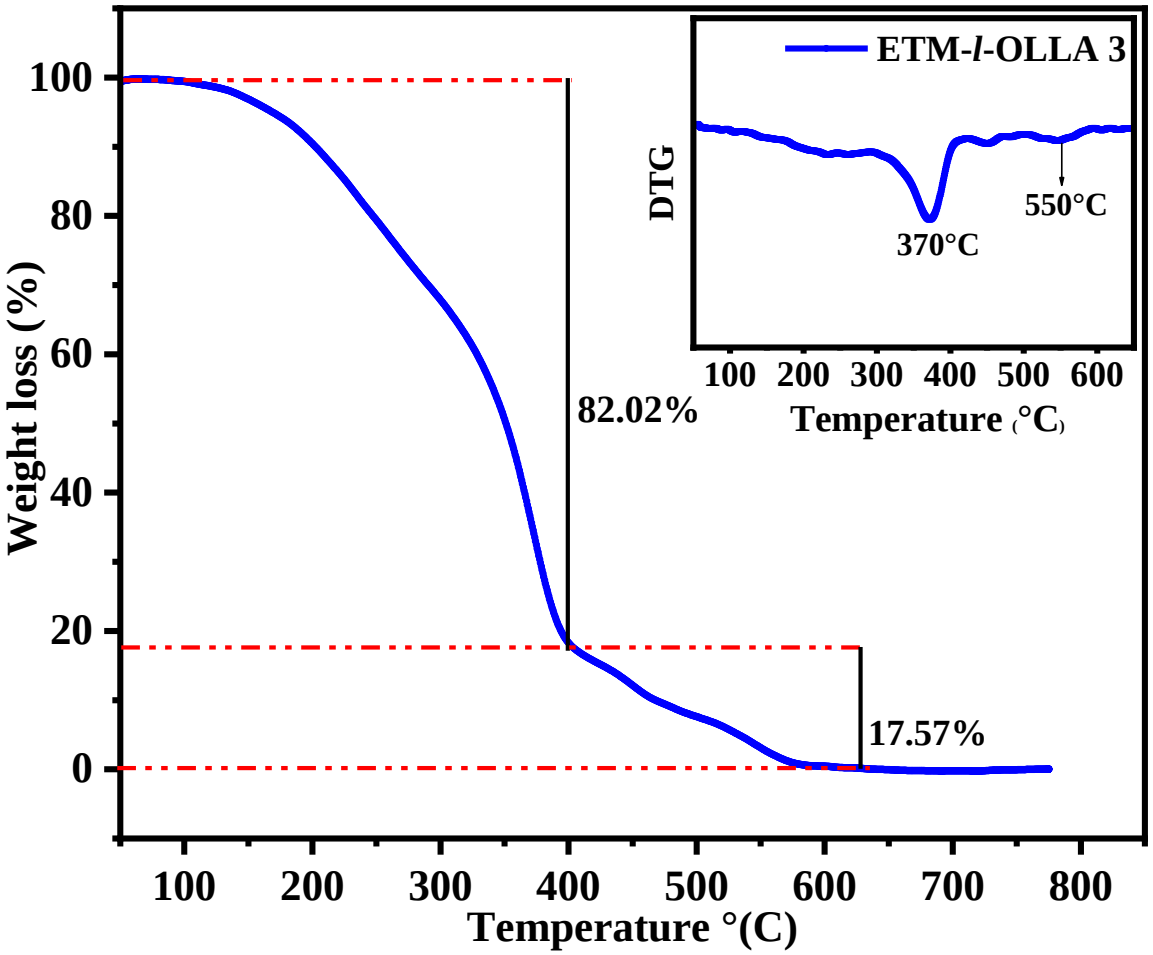


Figure 4. 17: TGA and DTG curve for ETM-*l*-OLLA-3

The weight loss of the EMT-*l*-OLLA bio-adhesive at different degradation temperatures can be summarized (Table 4.10). The EMT-*l*-OLLA 3 lost its 10% of weight at a temperature of 200°C where the main starting material that is ETM latex lost its 10% of weight at a low temperature which is 83°C. EMT-*l*-OLLA 3 bio-adhesive lost its 50% and 90% weight at a temperature of 383°C and 471°C respectively. And from the DTG curve, the maximum degradation temperature for EMT-*l*-OLLA 3 is 370°C whereas the ETM latex was 100°C. The maximum degradation temperature for the OLLA was 324°C (appendix 4). The synthesized ETM-*l*-OLLA bio-adhesive has improved thermal stability than that of the starting material.

Table 4. 10: TGA analysis of EMT-*l*-OLLA bio-adhesive, ETM latex, OLLA

Samples	**The Temperature at Different Weight Loss (°C)**			
	T_{10}	**T_{50}**	**T_{90}**	**T_{max}**
OLLA	211	309	347	324
ETM latex liquid	83	107	372	100
EMT-*l*-OLLA-3	200	383	471	370

4.6.5. Anti-Bacteria Activity

The in *vitro* antibacterial activity of the synthesized ETM latex-lactic was investigated at three different concentrations 1, 2, and 3mg/mL against one Gram-negative bacteria and one Gram-positive bacteria with agar disk diffusion assay. The solution was poured to approximately 20 mL of sterile MHA in sterile culture plates and allowed to attain room temperature.

In parallel to this investigation, the lactic acid oligomer and the freshly trapped *Euphorbia trigona* Mill latex were also tested for their antibacterial effect and the result was used to confirm the effect of the synthesized adhesive. Mueller–Hinton sterile agar plates were seeded with indicator bacterial strains (1.5×10^8) and allowed to stay at 37°C for 3 hr. Sterile filter paper disks with a diameter of 6 mm were placed over these plates. Finally, the plates were incubated at 37°C for 24 hr. The inhibition zones were measured with a ruler and compared with the positive control disk (disk containing ampicillin) of the same concentration in *Mill*imeter. DMSO was used as a negative control during the whole test.

L-Lactic acid oligomer: With a similar test procedure to that of the adhesive synthesized the lactic acid oligomer was tested for its antibacterial effect against the two bacteria. From the zone of inhibition measurement, it was observed that the oligomer by its anti-bacteria effect with a zone of inhibition of 8mm for *E.coli* and 9mm for *S. aureus* with a concentration of 1mg/mL. It is almost comparable to the positive control used under this study that is 11mm and 12mm for *E.coli* and *S.aureus* this can be seen from the figure attached below (figure 4.18 and table 4.11).

***Euphorbia trigona* Mill latex**: The *Euphorbia trigona* Mill latex exhibited effective anti-bacterial activity for both bacteria. The latex obtained from Euphorbia species is known to be the most valuable product because it contains several biologically active natural compounds, such as triterpenoids. In this case, the main structure of the latex of *Euphorbia trigona* Mill is triterpenes, and this phytochemical compound is known to be a potential source of medicine (Salehi et al. 2019). The activity of this bioactive compound has different action mechanisms towards different bacteria, viruses, and also fungi. The pentacyclic triterpene being investigated in this study work can also have an action of a mechanism through the inhibitory mechanism for the biofilm-forming pathogens like *E.coli* and *S.aureus*. The anti-bacteria activity of this compound results from the hydroxyl, and carbon to carbon double bond (Chung 2020). As it can be seen from figure 4.18 and table 4.11 the zone of inhibition for the *Euphorbia trigona* Mill latex with 1mg/mL was found to be 12.5mm for *E.coli* which is greater than the control given as 11mm. Similarly for the *S.aureus* zone of inhibition was 12mm that is equal to the positive control.

The two bacterial strains the *Staphylococcus aureus* and *Escherichia coli* are known to be two common pathogenic bacteria that are wound-causing in humans' life. As a general, microbial-caused wound infections are becoming more common due to antibiotic-resistant strains proliferating, and the lack of and high cost of new generation antibiotics has continued to increase wound-related morbidity and mortality (Taye et al. 2011). In countries like Ethiopia, folk medicines are given a good awareness that involves using plant parts thus performing ethnobotanical study based on this can result in a way to find a substitute source of antibiotics. The *Euphorbia Trigona Mill* latex investigated under this study is documented as a medicinal constituent in our country (Wubalem, Tadesse, Getachew, Desalegn and Abraham 2012) and others like India (Nashikkar et al. 2011). The anti-bacteria activity found in this study for the ETM latex against those two bacteria's is comparable to the other experimental data, so utilizing this material as bio-medical substituent is possible.

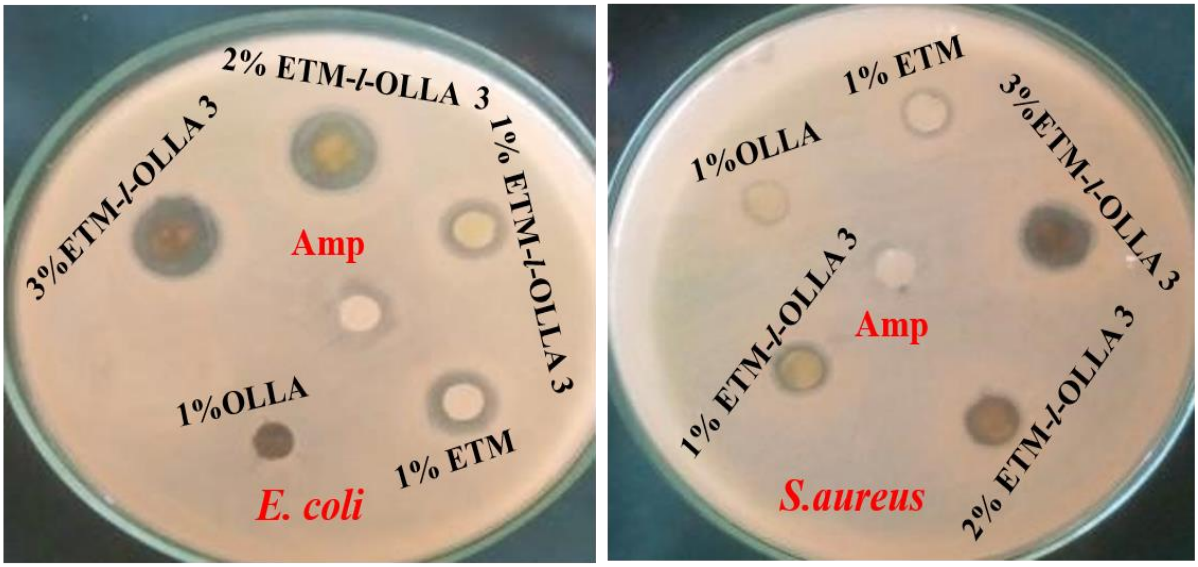


Figure 4. 18: Result for anti-bacteria analysis of ETM latex (1), lactic acid oligomer (2), ETM-*l*-OLLA-3 bio-adhesives (3)

Table 4. 11: Anti-bacterial activity of ETM latex, lactic acid oligomer and synthesize oligomer in a zone of inhibition (mm)

Cpd Conc.	**Zone of Inhibition(mm)**	
	***E. coli* ATCC25922**	***S. aureus* ATCC25923**
1%ETM	12.5	12
1%OLLA	8	9
1%ETM-*l*-OLLA 3	9	8
2%ETM-*l*-OLLA 3	13	11
3%ETM-*l*-OLLA 3	15	14
Ampicillin (+ ve control)	11	12

ETM-*l*-OLL-3 bio-adhesive: The bacteria activity of the ETM-*l*-OLLA 3 bio-adhesive was conducted for both two bacteria's *E.coli* and *S.aureus* with a similar test method to that of the ETM latex and L-Lactic acid oligomer. The ETM-*l*-OLLA 3 with a concentration of 1mg/mL show a zone of inhibition of 9mm and 8mm for *E.coli* and *S.aureus* bacteria's respectively. This inhibition effect for the bio-adhesive is slightly less than, that of ETM latex which is 12.5mm and 12 and also the positive control used having a zone of inhibition of 11mm and 12mm for the *E.coli* and

S.aureus. But when the concentration of the ETM-*l*-OLLA 3 bio-adhesive increase from 1mg/mL to 2mg/mL the zone of inhibition increases to 13mm and 11mm for *E.coli* and *S.aureus* respectively. A similar increment was observed again when the ETM-*l*-OLLA 3 bio-adhesive increases from 2mg/mL to 3mg/mL. Form this concentration change and increment in a zone of inhibition show the concentration-dependent effect of the ETM-*l*-OLLA 3 bio-adhesive. The anti-bacteria effect of this bio-adhesive can also be compared with the L-Lactic acid oligomer. With a similar concentration that is 1mg/mL the lactic acid oligomer exhibited 8mm and 9mm inhibition diameter for *E.coli* and *S.aureus* which is less than the control and also than that of ETM latex, this can be taken as that the antibacterial activity of the ETM-*l*-OLLA 3 bio-adhesive get its anti-bacteria activity more of from the ETM latex than that of the lactic acid oligomer. As a source of drug development, the activity of the naturally occurring pentacyclic triterpenes can be increased by conjugation of the parent molecule to other molecules, other than the anti-bacteria (Hodon et al. 2019).

4.6.6. Adhesive Nature Investigation of ETM-*l*-OLLA Bio-Adhesive

Adhesive Performance Test with Drag Method: The adhesive package width, storage, machine setting, and fabric beam width are the primary factors that influence the performance of the adhesive bandage during production. Convectional adhesive production usually consists of five major steps, starting with polymerizing the adhesive material and ending with molding the adhesive bandage by cutting the wide coated adhesive fabric. During this long space production, the combination of the listed production factors impacts the final performance of the adhesive tape. The peel strength test, which determines the adhesive strength of two bonded materials, can be used to monitor the efficiency of an adhesive bandage (El Gholmy 2019).

In this study, the adhesive bandage was made by hand, and the peel strength of the adhesive was investigated indirectly by measuring the mass that could be loaded on it. The mass and the mass that is before the first peel start was defined as the minimum mass that can be loaded on it, and the mass that causes the peel and completely detaches the adhesive bandage from the substrate was defined as the maximum mass load on it. The recorded mass can be converted into an object's weight by multiplying it by gravitational force, and the time that the adhesive bandage can be carried before detaching from the substrate can be used to compare adhesive performance. The

calculated weight of mass loaded on the developed adhesive bandage glued to a substrate as peeling for the ETM-*l*-OLLA-1 was 0.013N as a minimum weight and 0.112N as the maximum weight, so, the peeling strength for this matrix is the force in between those forces. This force was estimated in between 0.153N to 0.264N for ETM-*l*-OLLA-2. With a similar procedure, the peeling strength for ETM-*l*-OLLA-3 was found to be 0.264N to 0.346N. From this investigation, the peeling strength for the ETM-*l*-OLLA bio-adhesive increases as the mix ratio increases, that is the amount of the raw material ETM latex increases.

Table 4. 12: Summary of mass loaded for peeling test experiment

Samples	**Mass before peel starts(g)**	**Mass as the peeling started(g)**	**F_{min} (N)**	**F_{max} (N)**
ETM-*l*-OLLA-1	1.347	13.044	0.013	0.112
ETM-*l*-OLLA-2	15.65	26.96	0.153	0.264
ETM-*l*-OLLA-3	26.92	35.28	0.264	0.346

CHAPTER FIVE

5. CONCLUSION AND RECOMMENDATION

5.1. Conclusion

Under this research work, synthesis and characterization of lactic acid grafted ETM latex-based anti-bacterial bio-adhesive was performed. As main feedstock, the ETM latex was characterized in terms of a chemical structure and thermal property and found to be a kind of polymeric material consisting of high carbon chains (up to C-30) indicating that its main structural skeletal to be triterpene, a secondary metabolite having an anti-bacterial activity. The thermal property of the ETM latex was studied for both cured and liquid latex, and the cured ETM was found to be more stable than that of the liquid one. The difference in the thermal stability of the two materials resulted from the spontaneous coagulation of the ETM latex at room temperature when exposed to air.

Based on the preliminary investigation the polycondensation reaction was performed with L-Lactic acid (88%) and the synthesized bio-adhesive was investigated for different physical (bulk density, solubility) and instrumental tests (NMR and FT IR) together with titration method (detection of the carboxylic group as an acid value) to confirm and predict the reaction mechanism. Basically, the detection of the carboxylic group was performed to see the effect of reaction time, mix ratio, and reaction temperature on the reaction condition. 3hours of reaction time a temperature of 150ºC with a mix ratio of 1:3(lactic acid to ETM latex) was selected as a suitable reaction condition.

The NMR analysis with the FT IR confirmed the elastomeric nature of the synthesized bio-adhesive with the presence of cis-1, 4-polyisoprene units. The attachment of the lactic acid oligomer on the back-bone of the ETM latex has been confirmed also with the help of this instrumental analysis. The thermal property of the synthesized ETM-*l*-OLLA 3 was studied with thermogravimetric analysis and major degradation of 82.02% with the maximum degradation temperature of 370°C. The anti-bacterial study was also performed on the synthesized bio-adhesive and found to have an effective antibacterial activity that results from the basic reacting component ETM latex which is the well-known bio-active secondary metabolite.

The adhesion nature of ETM-*l*-OLLA was investigated with an experimental setup in the laboratory by loading a known amount of mass on it and estimating the peel strength of the ETM-*l*-OLLA bio-adhesive. The commercial adhesive bandage was cleaned with solvent and the ETM-*l*-OLLA was applied on and glued on a leather substrate then the mass loaded to peel the adhesive bandage from the leather substrate was recorded and multiplied with gravitational force to be reported as a peeling force. With this method, the peel strength of the developed ETM-*l*-OLLA bio-adhesive was found to increase with the increasing of the ETM latex. Similarly, the hydrophilic nature of the ETM-*l*-OLLA was also found to decrease with the increasing of the starting material ETM latex. The water contact angle investigation of ETM-*l*-OLLA gives a contact angle of 30.78°, 47.41°, and 53.83° for ETM-*l*-OLLA 1, 2, and 3 respectively.

5.2. Recommendations

This research work was intended to synthesize and characterize ETM latex-based elastomeric bio-adhesive by insitu polycondensation reaction of L-Lactic acid. The characterization of ETM-*l*-OLLLA included thermal property analysis, chemical structure determination, anti-bacterial activity test, and also physical tests like hydrophilic nature, bulk density, solubility in comparison with the raw materials ETM latex. As this research work is new additional works for the future can be considered for further investigations:

- 2D NMR, DSC analysis for the ETM latex,
- In vivo cytotoxicity, adhesion, and instrumental peel strength test for the synthesized ETM-*l*-OLLA elastomeric bio-adhesive,
- The ETM latex contains cis-1,4- polyisoprene unit can be used as a source of natural rubber,
- Instrumental peel strength test for ETM-*l*-OLLA bio-adhesive to more investigate the adhesive performance,
- The adhesion strength can be studied by adding metal catalysts to the system for more crosslinked structure and structural adhesive applications,
- Moreover, the ETM-*l*-OLLA have film-forming characteristics and also possess elastomeric nature, hence can be used as a toughening agent for ductile plastics.

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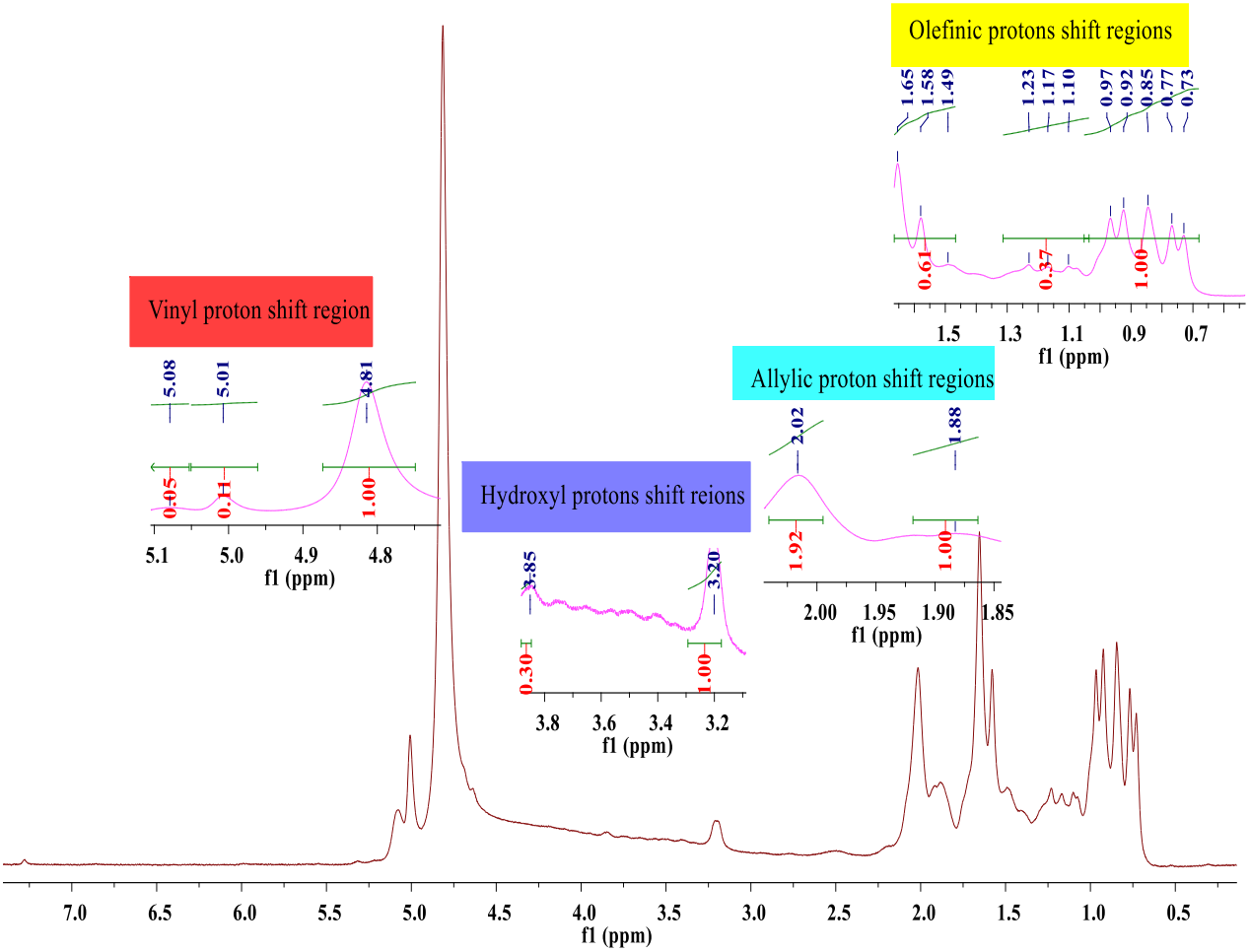
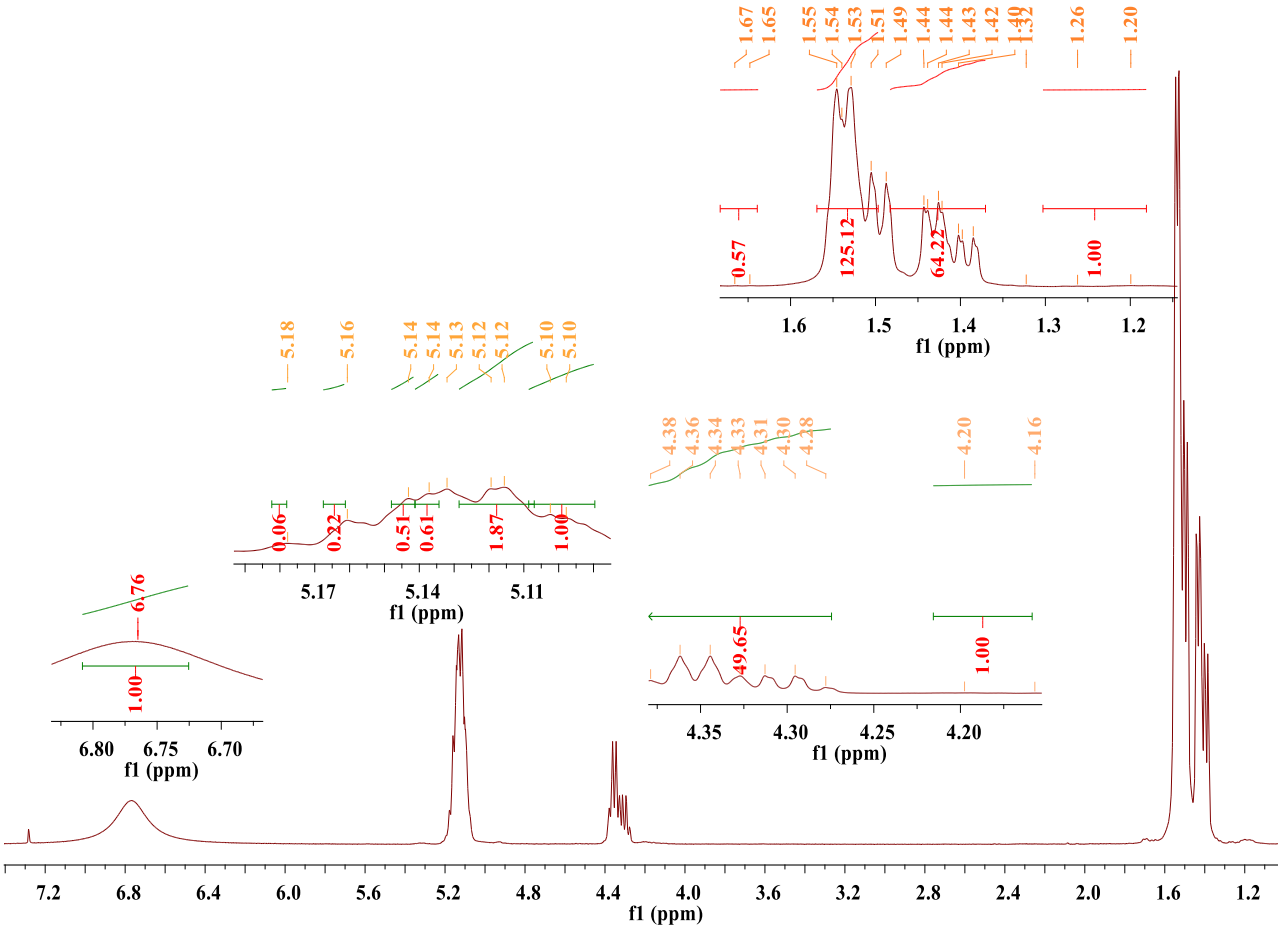
APPENDIXES

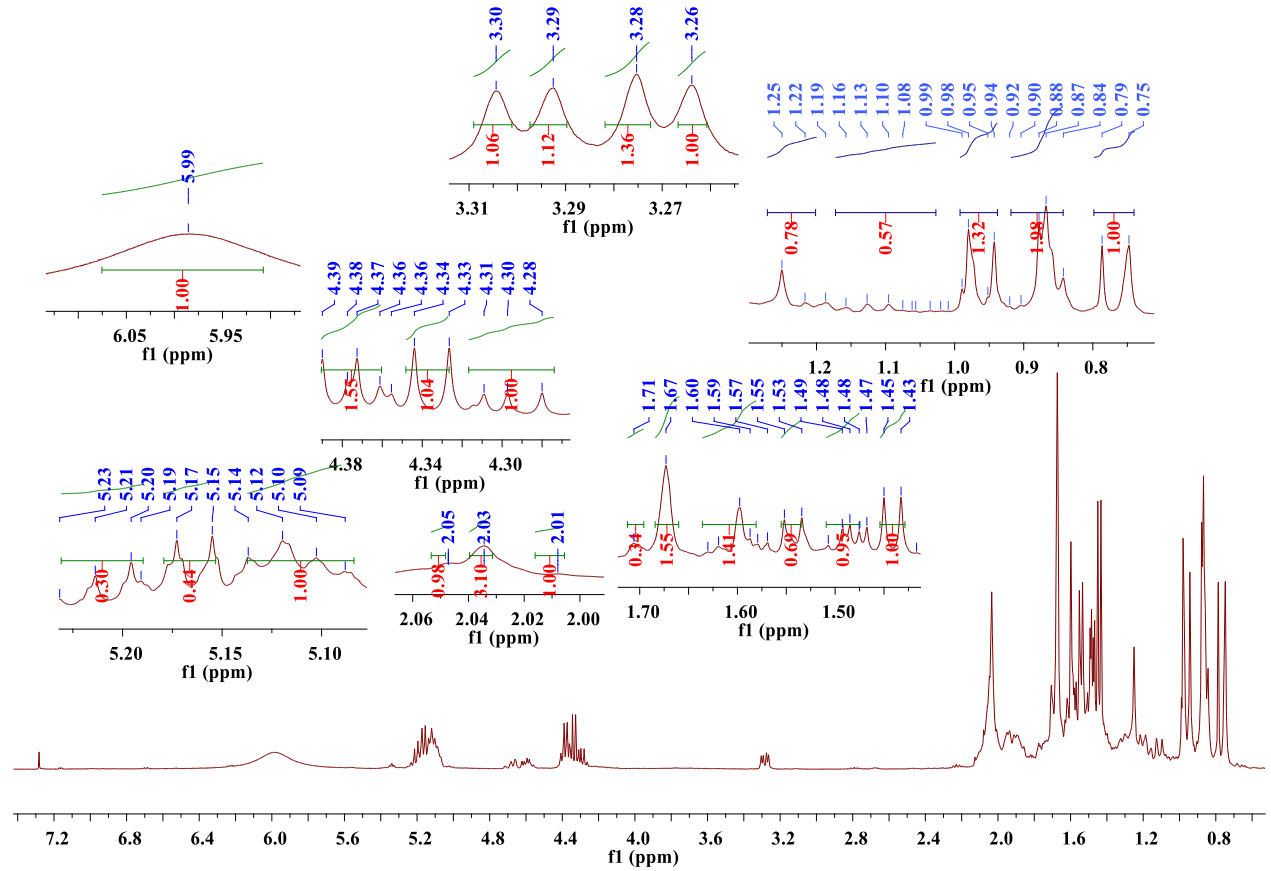
Appendix 1: 1H NMR of liquid ETM latex



Appendix 2: ^{1}HNMR of OLLA



Appendix 3: ^{1}H NMR of ETM-*l*-OLLA-3



Appendix 4: TGA and DTG curves for OLLA