Available at www.ijcasonline.com ISSN 2349 – 0594

International Journal of Modern Chemistry and Applied Science

International Journal of Modern Chemistry and Applied Science 2015, 2(4), 251-265

Phytochemical investigation on the Ethanol extract of the aerial parts of *Laggera tomentosa*

Yilma Hunde[1] and Nigist Asfaw[2]

[1]Chemistry Department, Ambo University, Ethiopia), [2]Chemistry Department, Addis Ababa University, Ethiopia

Corresponding author's email address: elema14@yahoo.com

Abstract: Laggera tomentosa in the family of Asteraceae, is a species endemic to Ethiopia. It has medicinal values and is important in the traditional medicine like the other species in the genus. In this work, one sesquiterpene and one flavone, namely 3-(3'-acetoxy-2'-hydroxy-2'-methylbutyryl)cuauhtemone (LTE-1) and 4',5,7-trihydroxy-3',3,6-trimethoxyflavone (LTE-2) were isolated from the aerial parts of the plant, respectively. LTE-1 was isolated before from the same plant and other species of Laggera. LTE-2 was reported before from Jasona montana plant growing in Egypt and Mentha royleana with the name Jaceidin. However, it was isolated for the first time from L. tomentosa. The structures were elucidated based on NMR and UV spectra and by comparison of the data obtained with those reported for related compounds in the literature.

***Keywords*-**Asteraceae, Flavone, Laggera tomentosa, Medicinal values, Sesquiterpene

1. Introduction

1.1. Natural products

Products of natural origins can be called "Natural products". Natural products include,[1]

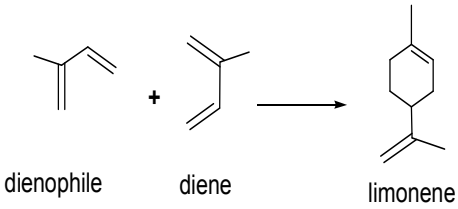
1) an entire organism: plant, animal, or microorganism that has not been subjected to any kind of processing or treatment other than a simple process of preservation, example: drying
2) part of an organism, examples: leaves, or flowers of a plant, an isolated animal organ
3) an extract of an organism or part of an organism or exudates and
4) pure compounds, examples: terpenes, flavoniods, alkaloids, coumarines, glycosides, lignans, steroids, sugars....,etc.isolated from natural sources.

Secondary metabolites refer to small molecules of natural products that are not necessary for their essential biochemical events. Even though the distinction between primary and secondary metabolites is often difficult, secondary metabolites are often species dependent.[1,2] Individual secondary metabolites may be common to a number of species or may be produced by only one organism.[3] Why plants produce secondary metabolites is still largely unknown and subjected to speculation. In many cases, the importance of a particular substance to the plant is not known. It has often been suggested that the plant simply excretes part of its waste products in the form of natural products. This is not an appealing suggestion since the natural products often exhibit very complicated structures. Recent development in biological science has given us some hints in understanding the importance of these compounds. Many natural products have a regulatory role (example, growth hormones). Some function as chemical defense agents against diseases. The role of certain compounds is to act as chemical messenger molecules between species of the same genus. A large number of new chemical entities are arrived at through the help of natural products. Our interest in natural products can be traced back thousands of years for their usefulness to humankind, and this continues to the present day. The ability to access natural products, understand their usefulness and drive applications, has been a major driving force in the field of natural product research. Natural products have played a great role in the development of medicinal chemistry. Natural product chemistry covers the chemistry of naturally occurring organic compounds: their biosynthetic pathways, function in their own environment, metabolism and more conventional branches of chemistry such as structural elucidation and synthesis. Natural products played a prominent role in ancient traditional medicine systems that are still in common use today. Of the roughly 350,000 species of plants believed to exist, one-third of those have yet to be discovered. Out of the quarter million that have been reported, only fractions of them have been chemically investigated.[20] According to the WHO, 75% of people still rely on plant based traditional medicines for primary health care globally. So, in recent years a significant revival of interest in natural products as a potential source for new medicines has been observed among academic as well as pharmaceutical companies.[1] Therefore, the main objective of this work was the phytochemical investigation on the ethanol extract of the aerial parts of Laggera tomentosa and elucidation of structures of chemical constituents of the plant. This plant was selected for this study because it is endemic to Ethiopia and has traditionally medicinal values. Furthermore, there are no published reports on the solvent extracts of the plant except on the composition of the essential oil. Few compounds have been isolated from the solvent extracts of the plant before.[3,15,19] This work is a continuation of the previous research conducted by other graduate students at the department of Chemistry, Addis Ababa University (AAU).

1.2. Terpenes

In the early history of natural product chemistry, many strongly odorous plant compounds were observed to be formed from C_5 units called isopentenyl or isoprene units. These compounds were termed terpenes, the term was derived from the

terebinth tree, *Pistacia terebinthus*. Formally, terpenes are derived from isoprene units by joining two or more units from either end, the head or the tail. Thus, for example, limonene can be synthesized by a formal Diels-Alder reaction by joining the head of one isoprene unit with the tail of another one[4] (Scheme 1).



Scheme 1. Head-to-tail reaction of isoprene units

The diverse family of natural products constructed from five carbon building units and so comprising compounds with C_5, C_{10}, C_{15}, C_{20}, C_{30}….,etc. skeletons are synonymously termed terpenes, terpenoids or isoterpenoids, with the important subgroup of steroids and carotenoids. There is no agreement on the basic nomenclature and the various subgroups are often given the –iod or –ene suffixes interchangeably. For instance monoterpenes= monoterpenoids.[8] Terpenes are classified according to the number of isoprene units involved in their biosynthesis.[5] Monoterpenes, C_{10}; sesquiterpenes, C_{15}; diterpenes, C_{20}; sesterterpenes, C_{25}; triterpenes, C_{30} …., etc. (Figure 1). Often one or more carbon atoms are excised from the molecule, and these terpenes are indicated by the prefix *nor*: For example, norditerpene, C_{19} containing terpene.[4]

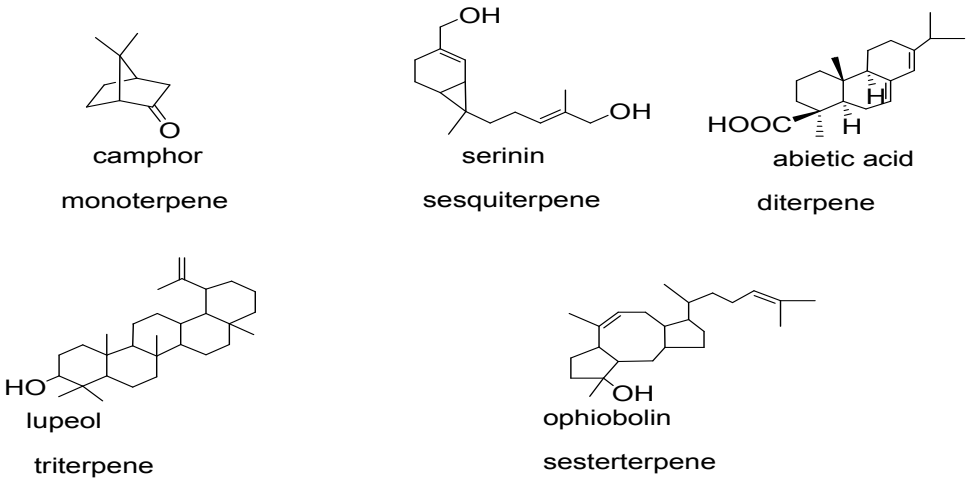


Figure 1. Some classes of terpenes

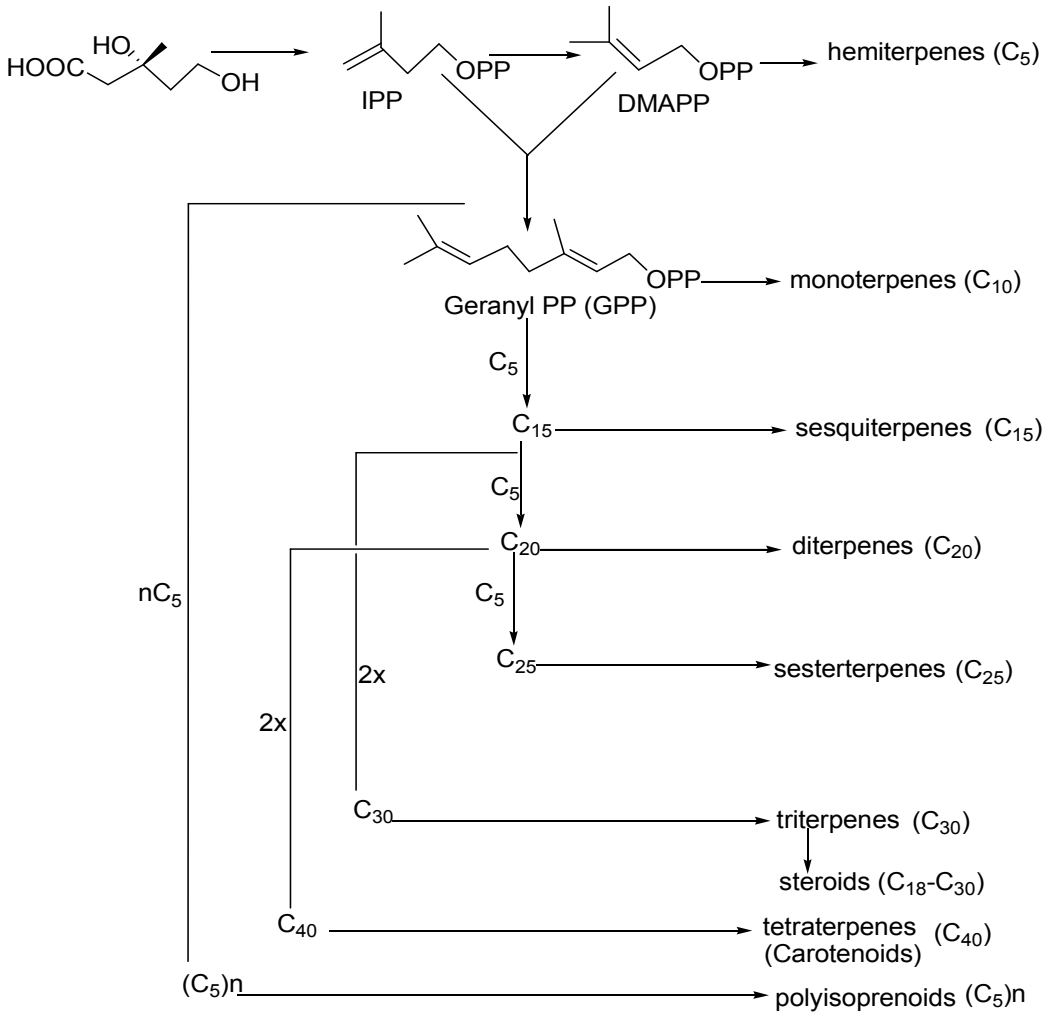
The terpene skeletons occur as open chain as well as in various cyclic forms. For example, addition of a C_5 IPP unit to geranyl diphosphate in an extension of the prenyl tranferase reaction leads to the fundamental sesquiterpene precursor, farnesyl diphosphate (FPP). FPP can then give rise to linear and cyclic sesquiterpenes. Because of the increased chain length and additional double bond, the number of possible cyclization modes is also increased, and a huge range of *mono*-, *bi*-, and *tri*-cyclic structures can result. The stereochemistry of the double bond nearest the diphosphate can adopt an E configuration (as in FPP) or a Z configuration via ionization, as found with geranyl PP [6] (Table 1).

The terpenes constitute the largest class of natural products [7] and have diverse applications in industry [5] (Table 2). Many familiar fragrances are terpenes with relatively small size and high volatility. For instance, the odour typical to lemons mainly owns to limonene [4], and the distinctive aroma of coniferous plantations is the result of the emission of volatile compounds such as α-pinene.[5]

1.2.1. Biosynthesis of terpenes

Terpenes are secondary metabolites synthesized by plants, marine organisms. For instance, bromo and chloro-substituents in algal terpenes, isonitrile and isothiocyanate substituents in sponge terpenes and protoilludane and cyathins in fungi by head to tail joining of isoprene units, isopentenyl pyrophosphate (IPP) parent i.e. hemiterpenoid.[5] This unit itself does not function as the reactive biogenetic species. The important reactive species involved in the formation of terpenes are isopentenyl and dimethylallyl pyrophosphates. These are formed from the mevalonic acid by phosphorylation followed by ATP-assisted loss of water and carbon dioxide to give isopentenyl pyrophosphate (IPP). Isomerization of the double bond by the catalytic action of IPP-isomerase gives dimethylallyl pyrophosphate (DMAPP) [4] (Scheme 3).

Mevalonic acid, 3R (+)-isomer, a C_6-acyclic compound, is the precursor of all terpenes. The parents of the various subclasses are, hemiterpenes from isopentenyl pyrophosphate and 3,3-dimethylallyl pyrophosphate (DMAPP), monoterpenes from geranyl pyrophosphate (GPP), sesquiterpenes from 2E,6E-farnesyl pyrophosphate (FPP), diterpenes from 2E,6E,10E-geranyl geranyl pyrophosphate (GGPP), sesterterpenes, from 2E,6E,10E,14E-geranyl farnesyl pyrophosphate (GFPP), triterpenes from squalene and carotenoids from phytoene. This implies that the central pathway up to C_{25} compounds is formed by sequential addition of C_5 moities derived from IPP to a starter unit derived from DMAPP. The parents C_{30} and C_{40} compounds are formed by reductive coupling of two FPP, i.e., C_{15} residues or GGPP, i.e., C_{20} moities respectively [8] (Scheme 2).



Scheme 2. Biosynthesis of terpenes

Table 1. Some skeletal types of terpenes [7]

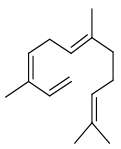
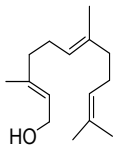
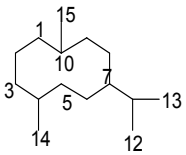
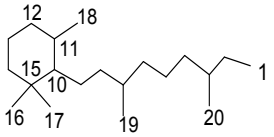
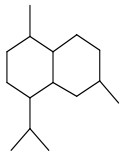
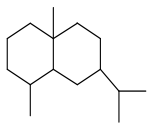
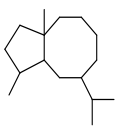
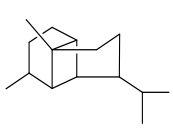
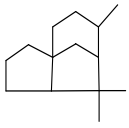
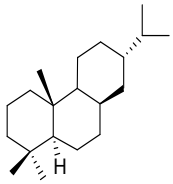
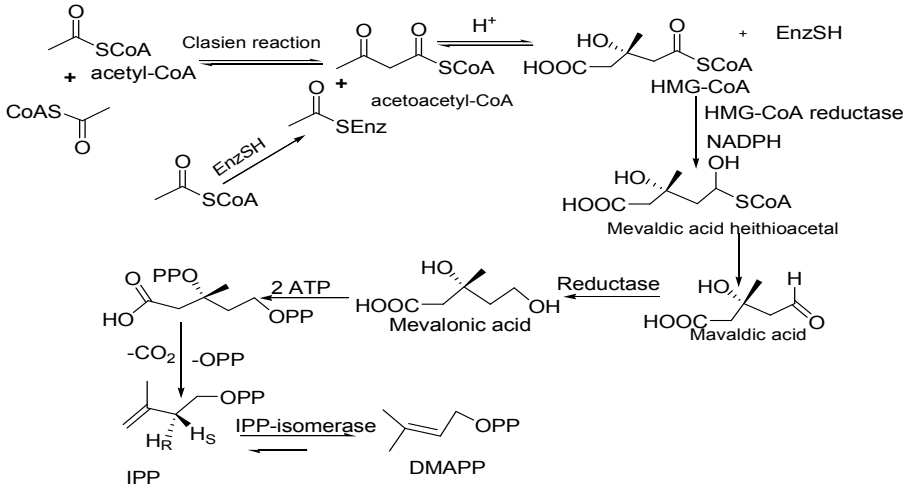
Skeleton	Compounds	
Acyclic	 farnesene	 farnesol
Monocyclic	 Germacrane	 Retinane
Bicyclic	 Cadinane	 Eudesmane
		 Guiane
Tricyclic	 Copane	 Cerdrane
		 Ambietane

Table 2. Industrial application of some terpenes

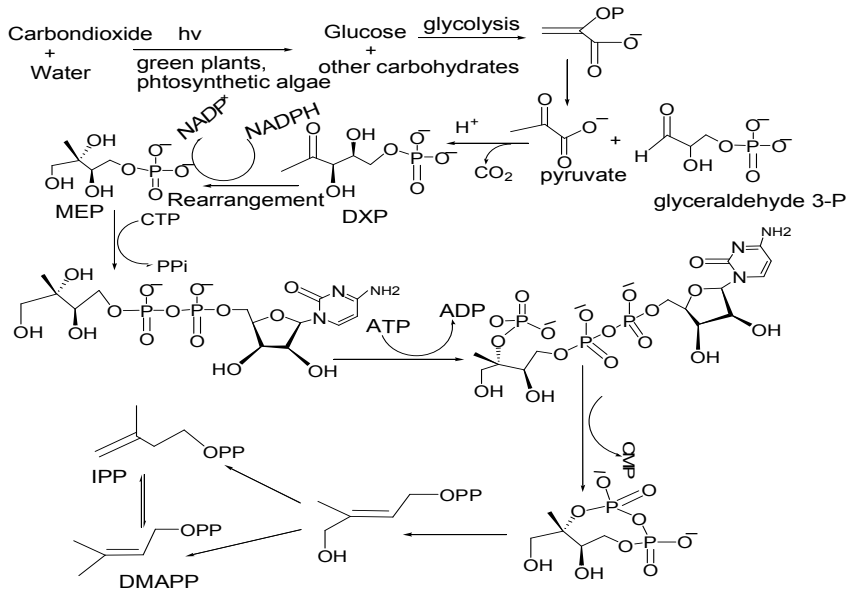
Terpenes	applications
Linalool	in perfumery
Citral	as mosquito repellant as starting material in Vit-A synthesis
Menthol	in pharmaceutical industries
Artemisinin	in pharmaceutical industries for anti-malarial synthesis
Farnesol & Juvabione	insect juvenile hormone
Gibberellic acids & Brassinolids	plant growth stimulators
Salannin & Azadirachtins	insect anti-feedant and growth inhibitors
Taxol & cucurbitacins	anti-tumor

Two pathways may derive the biochemical unit, i.e., isoprene unit: mevalonate pathway and deoxyxylulose phosphate pathway. In the mevalonate pathway three molecules of acetyl CoA are used to form mevalonic acid. Two molecules combine initially in a Claisen type reaction to give acetoacetyl CoA, and a third is incorporated through a specific aldol addition giving the branched chain ester β-hydroxy-β-methylglutaryl-CoA (HMG-CoA). Mevalonate is then transformed to IPP by phosphorylation twice at C_5 followed by decarboxylation step [6] (Scheme 3).

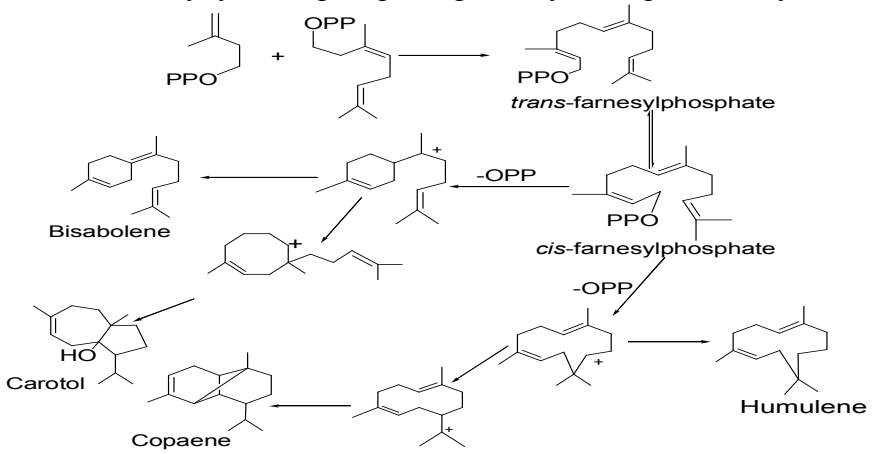


Scheme 2. Mevalonate pathway of isoprene biosynthesis

Deoxyxylulose phosphate pathway begins with conversion of glucose to glyceraldehyde-3-phosphate (GAP) and pyruvate, followed by thiamine-mediated decarboxylation of pyruvate. Condensation with GAP generates 1-deoxy-D-xylulose-5-phosphate (DXP). DXP then undergoes a rearrangement and red-uction to give 2-C-methyl-D-erythritol-4-phosphate (MEP). After several transformations, the cyclic diphosphate is made. The $[4Fe\text{-}4S]^{2+}$ metal cluster sequentially transfers two electrons to open the diphosphate and eliminate the inactivated secondary hydroxyl group. Similarly, another iron-sulphur cluster performs a second two electrons transfer to yield an allylic anion that can afford either DMAPP or IPP upon protonation [6] (Scheme 4).Sesquiterpenes are generally present in many plant species but especially more concentrated in plants yielding volatile or essential oils. They are formed from three isoprene units and thus contain 15 carbon atoms. The sesquiterpenes are formed from *cis-trans*-farnesyl pyrophosphate through cationic cyclization similar to the formation of the menthane cation (Scheme 5).[5] New sesquiterpenes and their lactones are being found at a surprising rate. For example, there were 1300 sesquiterpenes and their lactone derivatives known in 1981 and 3200 in 1987. This phenomenon places sesquiterpenes among the largest classes of natural products. An advance in the understanding of the biosynthesis of this group together with the great structural variety makes these compounds of great value in chemotaxonomy.[7]



Scheme 3. Deoxyxylulose phosphate pathway of isoprene biosynthesis



Scheme 5. Biosynthesis of some sesquiterpenes

1.3. Flavonoids

The flavonoids (2-phenylbenzpyrone) are a large group of biologically active natural products, distributed widely in higher plants, but also found in some lower plants, including algae. [1] Many flavonoids are easily recognized as flower pigments in most angiosperm families. However, their occurrence is not restricted to flowers but includes all parts of the plant. The chemical structures of flavonoids are based on a C_{15} skeleton with ring-C bearing a second aromatic ring-B in position 2, 3, or 4. In a few cases, the six membered heterocyclic ring C occurs in an isomeric open form or is replaced by a five-membered ring. Various subgroups of flavonoids are classified according to the substitution patterns of ring-C. Both the oxidation state of the heterocyclic ring and the position of ring-B are important in the classification. Examples of the six major subgroups are given in Figure 3. Most of these (flavanones, flavones, flavonols, and anthocyanins) bear ring-B in position 2 of the heterocyclic ring. In isoflavonoids ring-B occupies position 3 (Figure 3).

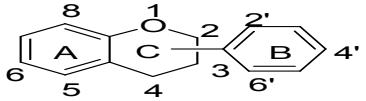


Figure 2. Basic structure of most flovonoids

Another small group comprises oligomeric flavonoids, biflavonyls, and proanthocyanidins. Altogether there are many differently substituted flavonoid aglycones. Most of these occur as glycosides with different combinations of sugars attached to hydroxyl groups. The sugars are often further substituted by acyl residues, such as malonate, 4-coumarate, caffeate, and ferulate. Some flavonoids occur as C-glycosyl derivatives in position 6 or 8. Flavonoids use as attractants of animals in fertilization process in higher plants. Other important functions are attributed to flavonoids as protective agents against UV-light or infection by phytopathogenic organisms. Flavonoids are often rapidly metabolized after synthesis.[9] A significant role of flavonoids that has been under very active research recently, is their possible beneficial influence on human health. There is growing evidence from human consumption studies supporting a protective role of flavonoids in cardiovascular diseases and cancer. Many flavonoids have been found to possess antiviral, antibacterial and antifungal properties. In vitro, flavonoids have been found to own potent antioxidant [3] and some flavonoids have shown strong enzyme inhibiting activities. [10]

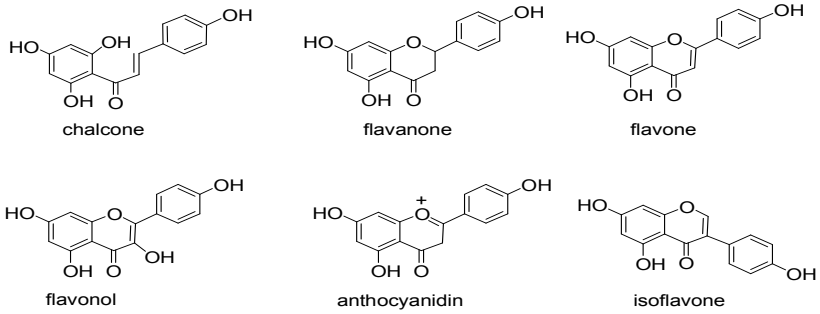
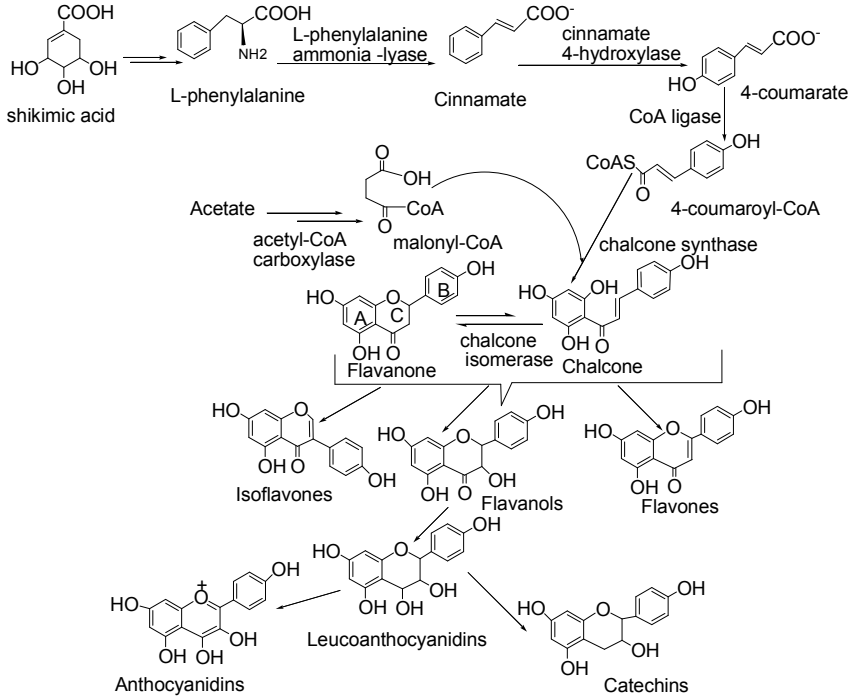


Figure 3. Representative examples of each of six major subgroups of flavonoids

1.3.1 Biosynthesis of Flavonoids

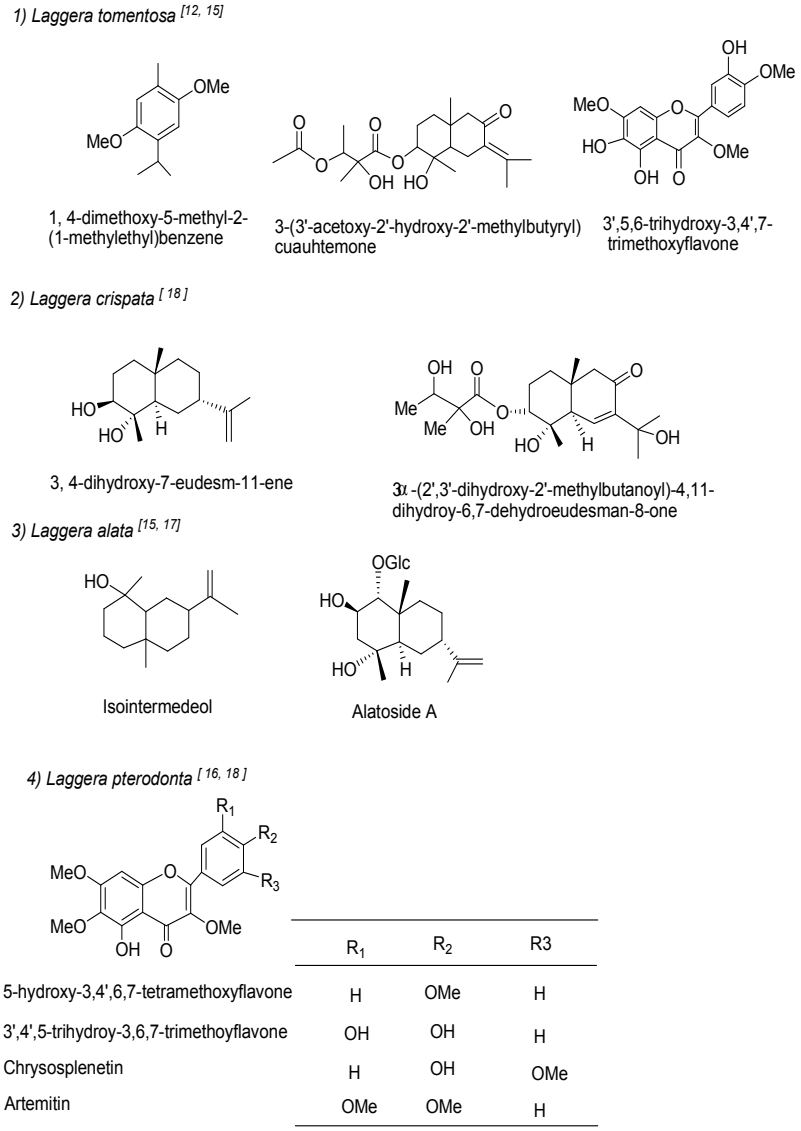
The early steps in the biosynthesis of the various subgroups of flavonoids are closely related. Earlier experiments with radioactivity labeled precursors established that the carbon skeleton of all flavonoids is derived from acetate and L-phenylalanine. Ring-A is formed from three acetate units, and shikimic acid gives phenylalanine that forms ring-B and C-2, C-3, and C-4 of the heterocyclic ring-C. A central intermediate in the formation of all flavonoids is the chalcone or the isomeric flavanone. [9] More generally, it is presumed that all aromatic rings having *ortho* hydroxyl groups arise from shikimic acid and all aromatic rings with *meta* hydroxyl groups arise from acetate. They have also shown that C_6-C_3 compounds as L-phenylalanine, cinnamic acid and ferulic acid are efficient precursors of the C_6 (B)-C_3 portion of flavonoids [9] (Scheme 6).



Scheme 4. Common steps in the biosynthesis of flavonoids

1.4. Genus *Laggera*

The genus *Laggera* Sch. Bip.Ex. Benth. & Hook (Asteraceae) comprises about 17 species confined to the old world tropics.[12] About 6 species occur in the Flora of Ethiopia and Eritrea.[13] The Asteraceae is one of the largest families of vascular plants with about 1300 genera and 25,000 species.[14] In Ethiopia there are about 6 *Laggera* species, *L. tomentosa* Sch. Bip. Ex. Oliv., *L. crispata* (vahl) Heeper & Wood, *L. braunii* vatke, *L.elatior* R.E. Fries, *L. crassifora* Sch. Bip. Ex. Rich. Oliv. & Hern and *L. alata* (D.Don) Sch. Bip. Ex. Oliv.[13] A number of *Laggera* species have been widely used in traditional medicine in south east Asia and Africa. For example, *L. pterodonta* (DC) Benth (Asteracea) is traditionally used as anti-inflammatory and antibacterial by the natives in south western China. Pharmaceutical testing has also shown that the plant possesses anti leukaemia activity as well as to inhibit experimental acute bronchitis.[16,24] *L. alata* var. alata Sch. Bip. Ex. Oliv. is widespread in the highlands and east coast part of Madagascar and has some tradition medicinal values including the use of its volatile components as an antiseptic.[17] *L. decurrens* vahl Hepper & Wood is quite common in Somalia and Southern Africa and is well known for its use in traditional medicine. In Namibia, extract of the leaves and roots of *L. decurrens* is drunk to relief stomach pains.[15] Recently, much attention has been given to *Laggera* species and their chemical contents because of their extensive activities. Some chemical constituents of *Laggera* species are given below in Figure 4.



	R_1	R_2	R3
a) 5-hydroxy-3,4',6,7-tetramethoxyflavone	H	OMe	H
b) 3',4',5-trihydroy-3,6,7-trimethoyflavone	OH	OH	H
c) Chrysosplenetin	H	OH	OMe
d) Artemitin	OMe	OMe	H

Figure 4. Some chemical constituents of *Laggera* species

1.5. *Laggera tomentosa*

Laggera tomentosa is a bushy perennial herb or subshrub, (0.5-1.2 m high) aromatic, narrowly winged, wings continuous, c1-1.5 mm wide, densely tomentose, ashy green or grey leaves[13] known in Oromo language as "Keskese" and endemic plant to Ethiopia.[12] It is found in Tigray, Gonder, Gojjam, Wollo, Shewa and Arsi on dry hill and mountain slopes at an altitude of 2345-2950 m high. It is a well-known and frequently cultivated medicinal plant. [13] The juice of the crushed plant is ingested as a treatment for stomach-ache and is used against migraine.[12] Its aerial parts are used as a treatment of tooth-ache, swelling and ringworm.[18] It can also be used as a fumigant and for cleansing milk containers.[13] Some phytochemical investigations on the essential oil of *L.tomentosa* have been reported before[12] and few compounds from the solvent extracts of the plant (Table 3) have been isolated before.[3,11,19]

2. Experimental

2.1. General

NMR (1H-NMR: 400 MHz, 13C-NMR: 100.60 MHz) spectra were measured on an Avance 400 Fourier transform spectrometer (Bruker). Chemical shifts were expressed in δ(ppm) and coupling constants (J) in Hertz (Hz). CDCl3 and acetone-d6 were used as solvent. The UV spectra were recorded on Spectroscopic Genesys 2PC UV-VIS scanning in the range 200-800 nm. The optical rotation was measured on Autopolo IV polarometer. TLC analysis was carried out on TLC plate 0.20 mm thick layer of merck silica gel 60 F254 coated on aluminium foil. Compounds on TLC were detected using UV-VIS light and spraying with 1% vanillin in sulfuric acid solution and heating.

2.2. Plant material

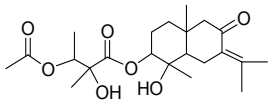
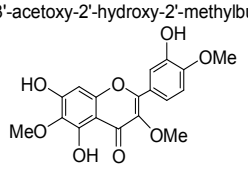
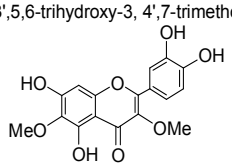
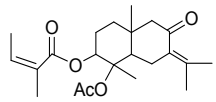
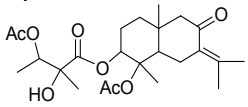
Laggera tomentosa was collected and identified by Prof. Sebsebe Demissew (Biology Department, AAU) from Daletti, South western shewa of Ethiopia on 22, February, 2009. A voucher specimen (SD 6487) is deposited at the National Herbarium (ETH.), department of biology, Addis Ababa University, Addis Ababa.

2.3. Coding system

In LTE, L- stands for the genus name Laggera, T- stands for the species name tomentosa and E- stands for the ethanol extract, and number-stands for the isolation order.

Laggera tomentosa (Photo from AAU garden by Yilma H.)

Table 3. Chemical constituents from solvent extract of *L. tomentosa*

Nº	Compounds	Solvent	Ref.
1	 3-(3'-acetoxy-2'-hydroxy-2'-methylbutyryl)-cuauhtemone		
2	 3',5,6-trihydroxy-3, 4',7-trimethoxyflavone	Ethanol	[15]
3	 3',4',5,7-tetrahydroxy-3,6-dimethoxyflavone		
4	 4-acetoxy-3-angeloyloxy-7,11-dehydroeudesman-8-one	Pet-ether	[19]
5	 4-O-acetyl-3-O-(2'-methyl-2'-hydroxybutyrate)-7,11-dehydroeudesman-8-one		

2.4. Isolation and Analysis

1kg of grounded aerial parts of the plant material was first soaked and extracted with 4 L petroleum ether (40-60 ^{0}c). The marc from the extract was then soaked with ethanol (3.5 L) for 3 days. The filtrate then was concentrated under a reduced pressure Rotary evaporator. The yield obtained was 68 g gummy black solid. 20 g of the crude from ethanol extract was applied on a silica gel (180 g) column chromatography and eluted with $CHCl_3$:EtOAc (9:1). The solvent system was gradually changed to high polarity ratio $CHCl_3$:EtOAc (2:3). 17 fractions were collected and TLC analysis was done. According to TLC results, these fractions were reduced to 3 fractions; F1, F2 and F3. This was done by comparison their Rf values, i.e., fractions with similar Rf values were mixed. Fractions F1, F2, and F3 were separately applied on sephadex LH-20 and eluted with $CHCl_3$:CH_3OH (2:1) and 10, 16 and 18 fractions were collected, respectively. Fractions 3 and 4 from F2 were mixed and applied on a silica gel (35 g) column chromatography and eluted with ethylacetate:pet-ether (7:3) and LTE-1 was isolated. Similarly, fractions 6-8 from F2 were mixed dried and the precipitate formed washed-well with pet-ether and LTE-2 was isolated (Scheme 7).

2.4.1. Isolation of LTE-1

F2 (fractions 5-10) from column chromatography was passed through sephadex LH-20 using $CHCl_3$:CH_3OH (2:1) as eluent and 16 fractions were collected. Fractions 3 and 4 were mixed and applied on a silica gel (35 g) column chromatography, using ethylacetate:pet-ether (7:3) as eluent afforded 25 mg LTE-1. It's TLC(Rf=0.40) run with ethylacetate:chloroform (1:1) showed pink color after

spraying with 1% vanillin in sulfuric acid solution and heating. The compound obtained was a yellow gummy solid. Optical rotation, $[\alpha]_D$= +58.60 (c=0.44, methanol, λ_{max}= 589 nm, T= 23 0c). The UV spectrum showed absorption maxima (in methanol) at 352.60 and 251 nm 1H NMR (400 MHz, CDCl3) δ:5.16 (1H, q, J=6.1 Hz, H-3'), 4.93 (1H, b, H-3), 2.96, 2.18 (1H, dd, J=4, 16 Hz, H-6), 2.25 (2H, s, H-9), 2.06 (3H, s,H-12), 2.01 (3H,s, H-7'),1.98,1.94 (1H, dd,J=4,16 Hz H-5),1.85 (3H,s,H-13),1.82 (2H, m, H-2),1.49,1.27 (2H, m, H-1), 1.43 (3H, s, H-5'), 1.31 (3H,d, J=6.1 Hz, H-4'), 1.29 (3H, s, H-15), 0.97 (3H, s, H-14). 13C-NMR (100.60 MHz,CDCl3) δ:202.03 (C-8), 174.84 (C-1'), 169.89 (C-6'), 145.63 (C-11), 130.51 (C-7), 78.99 (C-3), 76.32 (C-2'), 74.31 (C-3'), 72.30 (C-4), 59.84 (C-9), 46.82 (C-5), 35.95 (C-10), 33.34 (C-1), 25.49 (C-6), 23.87 (C-2), 23.60 (C-12), 22.88 (C-13), 22.35 (C-5'), 21.47 (C-15), 21.03 (C-7'), 18.60 (C-14), 13.31 (C-4').

2.4.2. Isolation of LTE-2

Fractions 6-8 from F2 formed precipitate. The TLC result showed almost single spot but with less polar minor impurity along with the major yellow spot after spraying with 1% vanillin in sulfuric acid solution. The dried precipitate was washed with the non-polar solvent pet-ether and TLC was checked for the pet-ether insoluble precipitate. This gave 22 mg pure LTE-2. It's TLC (Rf=0.66) run with ethylacetate:chloroform (1:1) showed yellow color after spraying with 1% vanillin sulfuric acid solution. The compound obtained was a yellow gummy solid and optically inactive. The UV spectrum showed absorption maxima (in methanol) at 358 and 271 nm. 1H NMR (400 MHz, acetone-d6) δ:7.79 (1H,d, J=2 Hz, H-2'), 7.71 (1H,dd, J=2, 8.6 Hz, H-6'), 7.02 (1H, d, J=8.6 Hz, H-5'), 6.61 (1H, s, H-8), 3.96 (3H,s, 3'-H-OCH3), 3.90 (3H, s, 3-H-OCH3), 3.89 (3H,s, 6-H-OCH3). 13C NMR (100.60 MHz, acetone-d6) δ:178.96 (C-4), 155.99 (C-2), 152.20 (C-7), 156.80 (C-9), 149.59 (C-4'), 147.40 (C-3'), 152.72 (C-5), 138.06 (C-3), 130.99 (C-6), 122.50 (C-6'), 121.99 (C-1'), 115.20 (C-5'), 111.77 (C-2'), 105.42 (C-10), 93.64 (C-8), 59.84 (C-6-OCH3), 59.35 (C-3-OCH3), 55.56 (C-3'-OCH3).

3. Results and Discussion

Two compounds, LTE- 1 and LTE-2 were isolated and characterized from the ethanol extract of *Laggera tomentosa*. Structural elucidation of the compounds was based on the spectroscopic data obtained for the compounds and in comparison with data in the literature for similar and related compounds. A flow chart that shows the separation scheme followed in the course of this work is given in scheme 7.

3.1. Characterization of LTE-1

LTE-1 is a pale yellow gummy solid with $R_{f=}$ 0.40. The UV spectrum displayed an absorption maxima at 352.20 and 251 nm (in methanol) indicated the presence of α,β-unsaturated carbonyl chromophore. ^{1}H-NMR spectrum (Table 4) of the compound showed a one-proton quartet at δ5.16 (*J*=6.1 Hz) indicating a methine attached to a methyl group and oxygen on the other side. A one-proton broad peak at δ4.93 showed the presence of methine attached to oxygen and carbon with chemically non-equivalent hydrogen groups on the other side. A one-proton singlet at δ3.49 indicated the presence of hydroxyl groups. The spectrum also indicated the presence of an acetate methyl group (δ2.01, 3H, *s*), quaternary methyl groups (δ1.29 and 0.97, 3H each *s*) and two olefinic methyl groups (δ2.06 and 1.85, 3H each *s*). Two other methyl groups (δ1.43, 3H, *s* and 1.31, 3H, *d*, *J*=6.1 Hz) were assigned to groups in an ester side chain. The doublet of doublet methine proton at δ1.98, 1.94 was attached to C-5 that is adjacent to a carbon with chemically non-equivalent hydrogens. Four methylene hydrogens were appeared at δ2.96, 2.18 (1H, *dd*, *J*=4, 16 Hz), 2.25 (2H, *s*), 1.82 (2H, *m*) and 1.49, 1.27 (2H, *m*). ^{13}C-NMR and DEPT-135 spectra indicated LTE-1 has 22 carbon atoms, eight quaternary, three methine, four methylene, and seven methyl carbons. The quaternary carbon atom at δ202.02 indicated the presence of conjugated carbonyl group. The quaternary carbon peaks at δ174.84, and 169.89 indicated two ester carbonyl groups. The peaks at δ145.63 and 130.51 indicated the presence of two olefinic carbon atoms. In addition, there were three quaternary carbon atoms at δ76.32, 72.30 and 35.95. The DEPT-135 NMR spectrum displayed four downward peaks at δ59.84, 33.33, 25.49 and 23.87, which revealed the presence of four methylene groups. There were ten peaks left which were assigned as three methine at δ78.99, 74.31, and 46.81, and seven methyl groups at δ23.60, 22.88, 22.35, 21.47, 21.03, 18.60, and 13.31 by comparing it with HMQC (Table 6). From the spectroscopic data obtained for the compound and by comparison with literature data, the following structure was proposed for the compound LTE-1 (Figure 5).

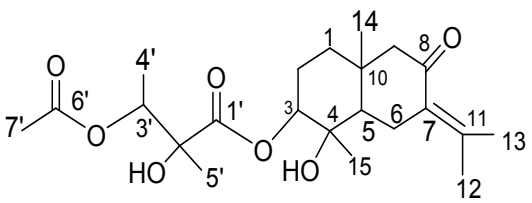
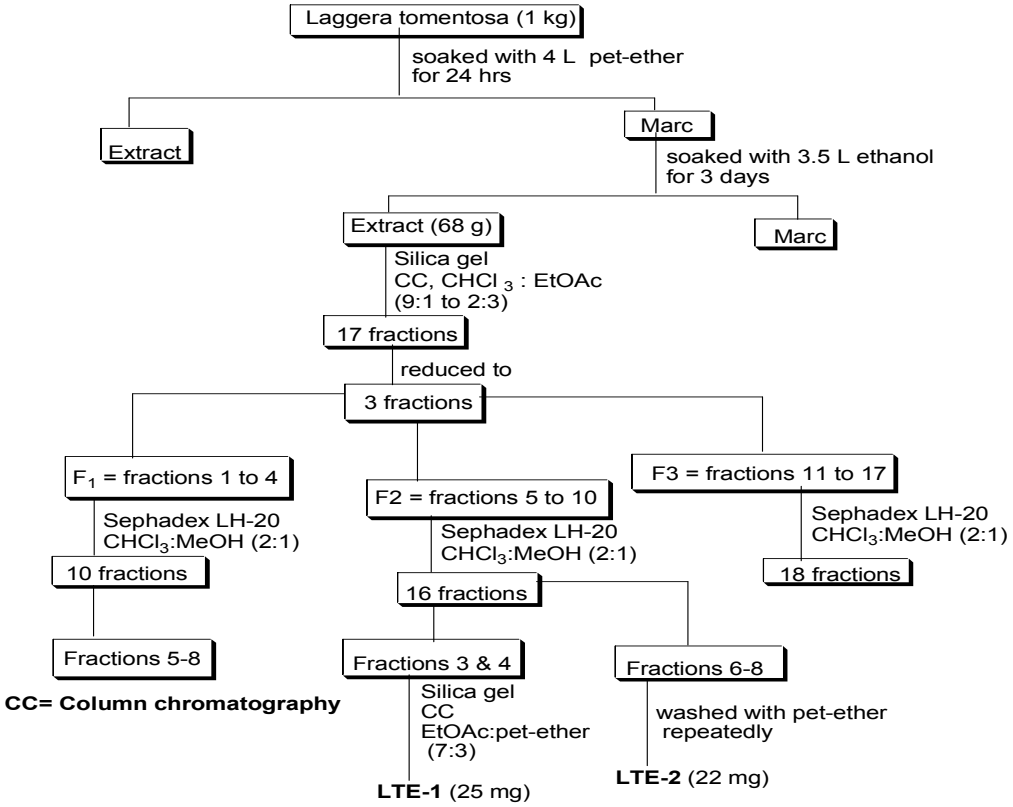


Figure 5. Proposed structure of LTE-1



Scheme 5. Flow chart of isolation of LTE components

Table 4. ^{13}C-NMR (110.60 MHz) and ^{1}H -NMR (400 MHz) data of compound LTE-1 (in chloroform-d, δ in ppm)

C N^{o}	^{13}C-NMR	^{1}H-NMR
1	δ33.34	δ1.49, 1.27(m)
2	23.87	1.82(*m*)
3	78.99	4.93(b)
4	72.30	-
5	46.82	1.98, 1.94(*dd*)
6	25.49	2.96, 2.12(*dd*)
7	130.51	-
8	202.03	-
9	59.84	2.25(s)
10	35.95	-
11	145.63	-
12	23.60	2.06(*s*)
13	22.88	1.85(*s*)
14	18.60	0.97(s)
15	21.47	1.29(*s*)
1’	174.74	-
2’	76.32	-
3’	74.31	5.16(*q*)
4’	13.31	1.31(*d*)
5’	22.35	1.43(*s*)
6’	169.84	-
7’	21.03	2.01(*s*)

Table 5. $^{1}H \longleftrightarrow {}^{1}H$ COSY correlation of LTE-1

C N^{o} (δ in ppm)	$^{1}H \longleftrightarrow {}^{1}H$ COSY
C-1 (δ33.34)	H-1a ⟷ H-1 a', H-2a, H-2 a'
C-2 (δ23.87)	H-2a ⟷ H-2 a', H-1 a, H-1 a'
C-3 (δ78.99)	H-3 ⟷ H-2a, H-2 a', H-15, H-1a
C-5 (δ46.82)	H-5 ⟷ H-6a, 6 a'
C-6 (δ25.49)	H-6a ⟷ H-6 a', H-5, H-9a, H-9 a'
	H-6 a' ⟷ H-6a, H-5, H-9a, H-9 a'
C-9 (δ59.84)	H-9a ⟷ H-6a, H-6 a', H-14
	H-9 a' ⟷ H-9a, H-6a, H-6 a',H-14
C-12 (δ23.60)	H-12 ⟷ H-13, H-6a, H-6 a'
C-13 (δ22.88)	H-13 ⟷ H-12, H-6 a',
C-14 (δ18.60)	H-14 ⟷ H-9a, H-9 a'
C-3' (δ74.31)	H-3' ⟷ H-4'
C-4' (δ13.31)	H-4' ⟷ H-3'
C-5' (δ22.35)	H-5' ⟷ H-3'

Table 6. HMQC correlation of LTE-1

C N^{o} (δ in ppm)	^{1}H (δ in ppm) & multiplicity
C-1 (δ33.34)	δ1.49 (2H, *m*) & δ1.27 (2H, *m*)
C-2 (δ23.87)	δ1.82 (2H, *m*)
C-3 (δ78.99)	δ4.93 (1H, *b*)
C-5 (δ46.82)	δ1.98 (1H, *dd*) & δ1.94 (1H, *dd*)
C-6 (δ25.49)	δ2.96 (1H, *dd*) & δ2.18 (1H, *dd*)
C-9 (δ59.84)	δ2.25 (2H, s)
C-12 (δ23.30)	δ2.06 (3H, *s*)
C-13 (δ22.88)	δ1.85 (3H, *s*)
C-14 (δ18.60)	δ0.97 (3H, *s*)
C-15 (δ21.47)	δ1.29 (3H, s)
C-3' (δ74.31)	δ5.16 (1H, *s*)
C-4' (δ13.31)	δ1.31 (3H, *d*)
C-5' (δ22.35)	δ1.43 (3H,*s*)
C-7' (δ21.01)	δ2.01 (3H, *s*)

In COSY spectrum, the protons at C-6 showed a strong correlation with the protons at C-5 due to a pair of diastereotopic protons at C-6 (δ2.96, 2.18). The predicted structure of compound LTE-1 is also supported by COSY spectrum Table 5 above. In HMBC spectrum (Table 7) a correlation appeared at δ18.60 with C-10, C-5 and C-1 indicated the position of C-14 to be on C-10. The presence ofα,β-unsaturated carbonyl group was confirmed by the correlation of protons on C-12 and C-13 with C-7 and C-11 (the olefinic carbons). Correlation of C-3 with the ester carbonyl carbon (C-1') indicated that the position of side chain to be at C-3 (Figure 7).

The 2D NMR spectra of LTE-1 further supported the proposed structure. The protons at δ4.93 (1H, *b*) and 5.16 (1H, q, *J*=6.1 Hz) were correlated with the carbon peaks at δ78.99 and 74.31, respectively and the proton peak at δ1.31 (3H, *d*, *J*=6.1 Hz) was correlated with the carbon peak at δ13.31 (Table 6). The spectroscopic data obtained for LTE-1 was found to be in agreement with the proposed structure for LTE-1. This compound was found to be identical to 3-(3'-acetoxy-2'-hydroxy-2'-methylbutyryl)-cuauhtemone which was previously isolated from *L. tomentosa*.[3, 15]

3.2. Characterization of LTE-2

LTE-2 is a yellowish solid with R_f=0.66. It was characterized as compound **3** based on spectroscopic data as described below. The UV spectrum showed absorption maximum at 358 and 271 nm (in methanol) were due to band I (range 300-550 nm) for ring-B cinnamoyl system and band II (range 240-285 nm) for ring-A benzoyl system, respectively. The ^{1}H-NMR spectrum displayed the presence of four protons in the aromatic region. The signal appearing at δ7.79 (1H, *d*, *J*=2 Hz), 7.71 (1H, *dd*, *J*=2, 8.6 Hz) and 7.02 (1H, *d*, *J*= 8.6 Hz) was due to an AA'B pattern of ring-B protons. The ^{1}H-NMR spectrum also showed a signal at 6.61 (1H, *s*) due to H-8, along with three signals for methoxy groups at δ3.96 (3H, *s*), 3.90 (3H, *s*) and 3.89 (3H, *s*). A broad singlet at δ12.99 confirmed the presence of hydroxyl functional group at C-5 that is chelated to the nearby carbonyl oxygen. Therefore, out of ten positions on the basic skeleton of flavonoid four positions were unsubstituted. Methoxy groups occupied the three positions. To decide the position of methoxy

substituents, 2D NMR (HMQC & HMBC) techniques were applied (Figure 9 and Table 10). In the HMBC spectrum, the downfield signal of the hydroxyl group at C-5 (δ12.99) showed correlation with the carbon signals at δ152.72 (C-5), 105.42 (C-10) and 130.99 (C-6). The aromatic protons at δ6.61 showed long-range connectivity with the δ152.17 (C-7), 156.80 (C-9), 130.99 (C-6) and 105.42 (C-10) which helped in assigning its position at C-8. The three aromatic protons with AA'B pattern could only be placed at ring-B. The signal at δ12.99 due to hydroxyl group indicated that C-5 position was occupied by hydroxyl functional group and thus AA'B system was not possible at ring-A.

Table 7. HMBC correlation of LTE -1

C N°.	HMBC
C-1	H-1→C-2
C-2	H-2→C-3
C-3	H-3→C-1, C-2, C-15,C-1'
C-5	H-5 →C-4, C-6
C-6	H-6→C-7, C-10
C-9	H-9 →C-1, C-5, C-7, C-8, C-10, C-14
C-12	H-12→C-7,C-11,C-13
C-13	H-13→C-8, C-11, C-12
C-14	H-14 →C-1, C-5, C-9, C-10
C-15	H-15→ C-3, C-4, C-5
C-3'	H-3'→C-1', C-4', C-5', C-6'
C-4'	H-4'→C-2', C-3'
C-5'	H-5'→C-1', C-2', C-3'

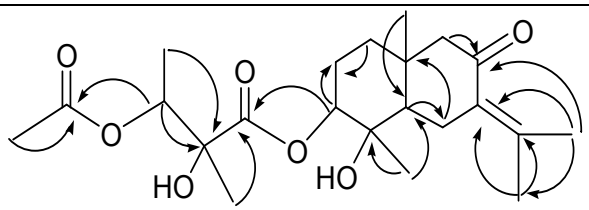
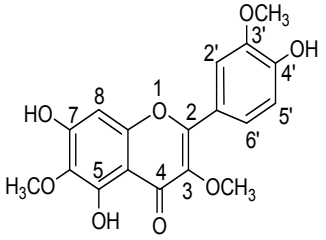


Figure 6. Important HMBC interactions of LTE-1

The HMBC (Table 10) of these protons confirmed the presence of a methoxy group on ring-B at δ147.40 (δ3.96). As the long-range correlation of H-6' proton did not reach till δ147.40 therefore a methoxy group was placed at C-3' and a hydroxyl group at C-4'. According to ^{13}C-NMR spectrum there were three methoxy, four methine, and eleven quaternary carbons. The DEPT-135 spectrum showed no signal due to methylene carbons. The ^{13}C-NMR chemical shift assignment was based on the use of flavone as a model.[21] All fifteen signals due to the flavones nuclei usually resonate in the region 90-200 ppm. The chemical shifts of the carbons of ring-C are usually distinct for flavones: C-2 (155-165), C-3 (136-139) and C-4 (176-184).[22] For the compound, LTE-2 the three carbons are found in the expected region. Methoxy carbons usually resonate at δ55-56.50 ppm. However, a down field shift to the range δ59.5-60.30 ppm is observed when the methoxy group is di-ortho substituted by substituent like hydroxyl, methoxy, or a ring junction. This confirmed LTE-2 to be a flavone.



Compound 3

Figure 7. Proposed structure of LTE-2

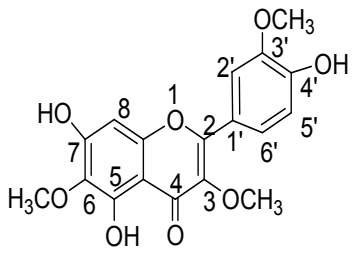


Figure 8. Structures of Jaceidin

Table 8. ^{13}C-NMR (100.60 MHz), ^{1}H-NMR (400 MHz) and DEPT-135 data of Compound LTE-2 (in acetone-d_6, δ in ppm)

C Nº.	^{13}C -NMR	^{1}H-NMR	DEPT-135
2	155.99	-	C
3	138.06	-	C
4	178.96	-	C
5	152.72	-	C
6	130.99	-	C
7	152.20	-	C
8	93.64	6.61 (1H, *s*)	CH
9	156.80	-	C
10	105.42	-	C
1’	121.99	-	C
2’	111.77	7.79 (1H, *d*)	CH
3’	147.40	-	C
4’	149.59	-	C
5’	115.20	7.02 (1H, *d*)	CH
6’	122.50	7.71 (1H, *dd*)	CH
OCH_3-6	59.84	3.89 (3H, *s*)	CH_3
OCH_3-3’	55.56	3.96 (3H, *s*)	CH_3
OCH_3-3	59.35	3.90 (3H, *s*)	CH_3
OH (C-5)	-	12.99 (1H, *br. s*)	-

Table 9. Comparison of the ^{1}H-NMR (400 MHz) and ^{13}C NMR (100.60 MHz) of LTE-2 with Jaceidin (Figure 9)

C Nº.	^{13}C (δ in ppm)		^{1}H (δ in ppm)	
	LTE-2	Jaceidin [10]	LTE-2	Jaceidin [10]
2	155.99	156.10	-	-
3	138.06	138.40	-	-
4	178.96	179.13	-	-
5	152.72	152.80	-	-
6	130.99	130.00	-	-
7	152.20	155.00	-	-
8	93.64	93.20	6.61 (s)	6.54 (*s*)
9	156.80	152.20	-	-
10	105.42	106.20	-	-
1’	121.99	122.60	-	-
2’	111.77	131.30	7.79 (*d*)	7.90 (*d*)
3’	147.40	127.42	-	-
4’	149.59	159.60	-	-
5’	115.20	110.30	7.02 (*d*)	6.98 (*d*)
6’	122.50	128.71	7.71 (*dd*)	7.99 (*dd*)
OCH_3-6	59.84	60.90	3.89 (*s*)	4.01 (*s*)
OCH_3-3’	55.56	55.60	3.96 (*s*)	3.91 (*s*)
OCH_3-3	59.35	60.10	3.90 (s)	3.82 (s)
OH (C_5)	-	-	12.99 (*s*)	12.94 (*s*)

The relative frequency of substitution for methoxy at C-3, C-6, and C-3’ and for hydroxyl at C-5, C-7, and C-4’ for flavones from plant family of asteraceae is also higher than the other alternative positions.[22] This information also more confirmed the proposed structure. Finally, based on the above spectroscopic and literature data, the structure of compound LTE-2 was proposed as 4’,5,7-trihydroxy-3’,3,6-trimethoxyflavone (Figure 7). This compound was reported from *Josana montana* a plant growing in Egypt and *Mentha royleana* with the name Jaceidin (Figure 8) before. [14] To our knowledge this is the first report of the compound from *L. tomentosa*.

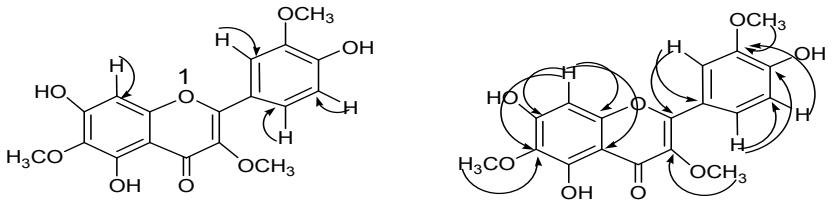


Figure 9. Important HMQC and HMBC interactions of LTE-2

Table 10. HMQC and HMBC correlations of LTE-2

HMQC		HMBC
C N^{o} (δ in ppm)	^{1}H (δ in ppm)	
C-8 (93.64)	6.61 (1H, s)	H-8 ⟶ C-2, C-6, C-7, C-9
C-2' (111.77)	7.79 (1H, d)	H-2' ⟶ C-2, C-1', C-3', C-4', C-6'
C-5' (115.20)	7.02 (1H, s)	H-5' ⟶ C-1', C-3', C-4', C-6'
C-6' (122.50)	7.71 (1H, dd)	H-6' ⟶ C-2, C-2',C-4'
6-OCH_3 (59.84)	3.89 (3H, s)	H-6-OCH_3 ⟶ C-6
3-OCH_3 (59.35)	3.90 (3H, s)	H-3-OCH_3 ⟶ C-3
3'-OCH_3 (55.56)	3.96 (3H, s)	H-3'-OCH_3 ⟶ C-3'

4. Conclusion

The chemistry of secondary metabolites of asteraceae has not been studied intensively over the last two centuries and several classification systems have been proposed based on combinations of chemical, morphological, and molecular data.[23] As a result, most species of the genus *Laggera* have received little attention even though they are rich in terpenes and flavonoids which have vital medicinal values. In this work, two compounds: one sesquiterpene, LTE-1 and one flavone, LTE-2 namely, 3-(3'-acetoxy-2'-hydroxy-2'-methylbutyryl)-cuauhtemone and 4',5,7-trihydroxy-3',3,6-trimethoxyflavone were isolated, respectively. LTE-1 has been isolated from *L. tomentosa* before and LTE-2 was isolated from *Jasonia montana* and *Mentha royleana.* However, LTE-2 was isolated for the first time from *L. tomentosa.*

5. Acknowledgments

I wish to express my profound gratitude to my advisor Dr. Nigist Asfaw for her interest, supervision and encouragement in the course of this work. I particularly express my deepest appreciation to Prof. Wendmagegn Mammo for his unreserved help and immediate response when support is needed and also for running the entire NMR spectrum and helping in structural interpretation of compounds and his encouragement throughout my work. My acknowledgment also goes to Prof. Sebsebe Damissew for his help in the collection and identification of plant material. I am thankful to Senait Dagne for her technical assistance, Kahil Kiros for his assistance and encouragement, Yoseph Atlaw for running NMR spectrum, Mr. Tsegaye Gudu for running UV spectrum, and Mr. Yirga Adugna for measuring optical rotation. Prof. Ermias Dagne is gratefully acknowledged for his help in allowing me to use ALNAP research laboratory for NMR and optical rotation services. I am also indebted to Dr. Pasham Venkatehswarlu for his unreserved assistance to shape my manuscript's contents. Finally, I express my deepest sense of gratitude to my lovely family and friends for their continuous encouragement during the course of this work.

6. References

1. Sarker, S., Latif, Z.,Gray, A., *Natural Products Isolation* (Totowa, New Jersey: Humana press, 2006).
2. Koskiner, A., *Asymmetric Synthesis of Natural Products* (Finland, University of Oulu, Oulu: John Wiley & Sons Ltd, 1993).
3. Haile, A., *Phytochemical investigation on the pet-ether extract aerial parts of L. tomentosa,* M. Sc. Project, AAU, Addis Ababa, Ethiopia, 2007.
4. Koskiner, A., *Asymmetric Synthesis of Natural Products*, (Finland, University of Oulu, Oulu: John Wiley & Sons Ltd, 1993).
5. Bhat, S., Nagasampagi, B., Sivakumar, M., *Chemistry of Natural Products*, 2007.
6. Dewick, P., *Medicinal Natural Products*, (United Kingdom, Nottingham: John Wiley & Sons Ltd, 2004).
7. Thomson, R., *The Chemistry of Natural Products*, (Great Britain, London: Falcon Graphic Art Ltd, 1993).
8. Mann, J., Davidson, R., Hobbs, J., Banthorpe, D. , HarBorne, J., Their Chemistry and Biological significance, in Edinburgh Gate (1st ed.), *Natural Products*, (England: Addison Wesley Longman Ltd., 1994) 161-176.
9. Stumpf, P. , Conn, E., *The Biochemistry of Plants*, (United Kingdom, London: Academic press, 2005).
10. Anis, I., Ahmed, S., Malik, A., Yasin, A., Choudary, M. I. , *Chem. Pharma Bull*., 515-518 , 50, 2002.
11. Robinson, T.,*The Organic Constituents of higher plants*, (North Amherst: Cordus press, 1975).
12. Asfaw, N., Storesund, J. H., Skattebol, L., Aasen, A. J., *Phytochemistry*, 1491-1494 52,1999.

13. Mesfin, T.,*The Flora of Ethiopia and Eritrea*, 140-143, 4(2), 2004.
14. Soliman, F. M., Moussa, M. Y., Abdallah, H. M., Othman, S. M., AJBAS,148-152 , 3, 2009.
15. Gebrehiwot, K.., *Phytochemical investigation on the ethanol extract aerial parts of L. tomentosa* , M. Sc. Project, AAU, Addis Ababa, Ethiopia, 2006.
16. Zhao, Y., Yue, J., Lin, Z., Ding, J., Sun, H., *Phytochemistry*, 459-464, 44, 1997.
17. Raharivelomanana, P., Bianchin, J., Ramanoelina, A. R. P., Rasoarahona, J. R. E., Faure, R., Cambon, A., *Phytochemistry*, 1085-1088, 47, 1998.
18. Geyid, A., Abebe, D., Debella, A., Makonnen, Z., Aberra, F., Teka, F., Kebede, T., Urga, K.,Yersaw, K., Biza, T., Haile-mariam, B., Guta, M. J., Ethnopharm., 421-427, 97, 2005.
19. Amenu, D., *Phytochemical investigation on the ethanol extract aerial parts of L. tomentosa*, M. Sc. Project, AAU, Addis Ababa, Ethiopia, 2006.
20. Butler, M. S., Newman, D. J., *Natural Compounds as Drugs*, 4-7,1, 2008.
21. Wenkert, E., Gottlieb, H. E., *Phytochemistry*, 1811-1816, 16, 1977.
22. Harborne, J., Mabry,T., *The flavonoids*: Advances in Research, (USA, New York: Champman & Hall,1982).
23. Calabria, L. M., Emerenciano, V. P., Ferreeira, M. J. P., Scotti, M. T., Mabry, T. J., *NPC*., 1-9, 3, 2006.
24. Li, S., Ding, J., Jiang, B., Na, B., *Phytochemistry*, 2035-2036, 49, 1998