SYNTHESIS AND EVALUATION OF LANTADENE A AND B ANALOGS AS ANTIBACTERIAL AGENTS

Project Report submitted in partial fulfillment of the requirement for the degree of

Bachelor of

Pharmacy

Under the Supervision of

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to

JAYPEE UNIVERSITY OF INFORMATION TECHNOLOGY

Jaypee University of Information and Technology

Waknaghat, Solan – 173234, Himachal Pradesh

TABLE OF CONTENTS

TOPICS	*PAGE NO.*
CERTIFICATE	**3**
ACKNOWLEDGEMENT	**4**
SUMMARY	**5**
LIST OF FIGURES	**6**
LIST OF TABLES	**7**
CHAPTER- 1 INTRODUCTION	**8-9**
CHAPTER- 2 REVIEW OF LITERATURE	**10-19**
2.1 Triterpenoids of lantana	**10-16**
2.2 Biological Activity of lantana triterpenoids	**16-17**
2.3 Toxicity of Lantana	**17-18**
2.4 Lantana in folk medicine	**18**
2.5 Utilization of Lantana	**18-19**
CHAPTER-3 Research Envisaged	**20**
CHAPTER-4 Material and methods	**21-27**
4.1 Plant collection	**21**
4.2 Extraction and isolation of partially purified lantadenes	**21**
4.3 Purification of lantadene A by recrystallization	**21-22**
4.4 Purification of lantadene B by recrystallization	**22**
4.5 Reduction of Lantadene A and B	**23**
4.6 Preparation of 22β-hydroxy-3-oxoolean-12-en-28-oicacid (5)	**25**
4.7 Synthesis of 3*β*-substituted and 22*β*-substituted olean-12-en-28-oic acids	**25-27**
4.8 *In vitro* antibacterial activity	**27-28**
CHAPTER-5 Results and Discussion	**29-31**
REFERNECES	**32-37**
BRIEF BIO-DATA OF STUDENTS	**38**

CERTIFICATE

This is to certify that the project report entitled "**Synthesis and Evaluation of Lantadene A and Lantadene B analogs as antimicrobial agents"** submitted by Mr. Abhay Thakur and Mr. Himanshu Chauhan to the Department of Pharmacy, Jaypee University of Information Technology, Waknaghat (Solan), in partial fulfillment of the requirements for the award of the degree of Bachelor of Pharmacy, is a bonafide record of work out by them under my supervision. This work has not been partially or wholly to any other University or Institute for the award of this or any other degree or diploma.

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DATE:

ABHAY THAKUR

HIMANSHU CHAUHAN

SUMMARY

Lantana camara L. (Verbenaceae), is one of the most noxious weeds of the world and it has imposed great threat to overall ecological balance. *Lantana* consists of various natural products but triterpenoids attracted the most attention of scientific world because of their toxicity and anticancer properties. In this study we synthesized a series of hydrophobic esters at the C-3 position of Lantadene A and Lantadene B. The structure of these esters was confirmed by combined use of spectroscopy and elemental analysis. Synthesized compounds were evaluated against Gram positive bacteria *S. aureus* and Gram negative bacteria *E. Coli.*

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Himanshu Chauhan

Date

Dr. Manu Sharma

Date:

List of Figures

Fig.No.	Title	Page No.
1	Red flowering variety of weed *Lantana camara* L.	8
2	Triterpenoids isolated from *Lantana camara* L.	12
3	Triterpenoids isolated from *Lantana camara* L. (Cont.)	13
4	Triterpenoids isolated from *Lantana camara* L. (Cont.)	14
5	Triterpenoids isolated from *Lantana camara* L. (Cont.)	15
6	Triterpenoids isolated from *Lantana camara* L. (Cont.)	16
7	Reduced Lantadene A and B from Lantadene A and B.	22
8	Scheme 1: Isolation of Lantadene and their reduction	24
9	Scheme 2: Synthesis of lantadene hybrid compounds	26

LIST OF TABLES

1. ***In vitro*** **antibacterial evaluation of compounds** **31**

CHAPTER -1

Introduction

Lantana camara L. (Verbenaceae), commonly known as wild or red sage, is the most widespread species of this genus, growing luxuriantly at elevations up to 2000 m in tropical, sub-tropical and temperate regions. [1] It has encroached upon vast expanse of land area including pastures, orchards, tea gardens forests and agricultural lands in tropical and subtropical parts of the world and has imposed a great threat to grazing livestock and overall ecological balance. It has been regarded as one of the ten most noxious weeds in the world. [1] The ingestion of plant foliage by grazing animals causes hepatotoxicity which is an important cause of livestock morbidity and mortality in lantana-infested regions. [2, 3]

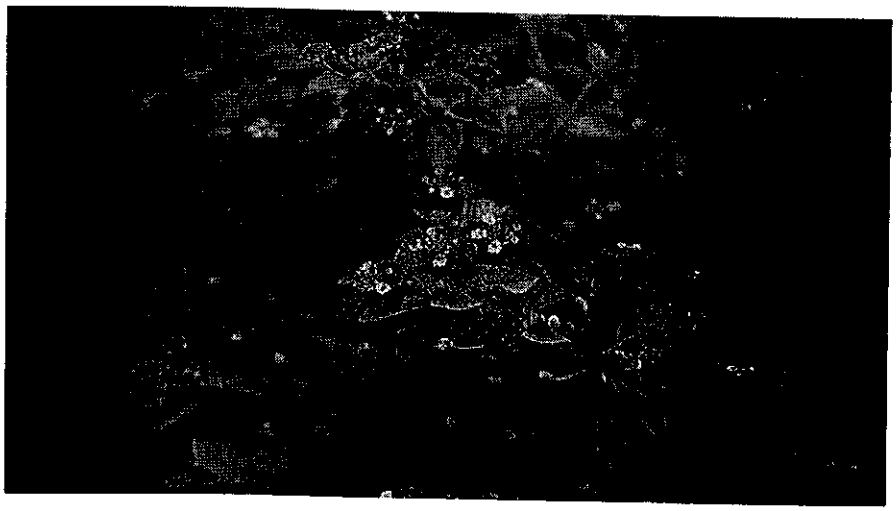


Figure 1: Red flowering variety of weed *Lantana camara* L.

Apart from its popularity as a weed, *L. camara* is said to form a useful hedge and to provide a good preparation for crops, covering the ground with fine leaf mulch. [4] It improves the fertility of rocky, grave, or hard laterite soils, enriches the soil and serves to retain humus in deforested areas and checks soil erosion. It can serve to nurse the parasitic sandalwood seedlings and in the Pacific islands has been used as a support for yam vines. *Lantana* leaves and twigs are often used in India as green mulch. The ash is rich in potassium and manganese which is useful in manuring coconut trees. The plant is not readily eaten by cattle unless pasturage is very scarce. In tropical countries, the ripe blue black berries are eaten, but ingestion of the green berry has led to human fatalities. [5, 6] Attempts to control this weed using mechanical, chemical and

biological methods have met with limited success and therefore, there is a need to find out some novel approaches for the utilization of this plant as a resource. A number of medicinal properties have also been reported of different parts of this plant. Various research groups have carried out systematic chemical investigations on lantana during the past few years and reported a number of chemical compounds with wide spectrum of biological activities.

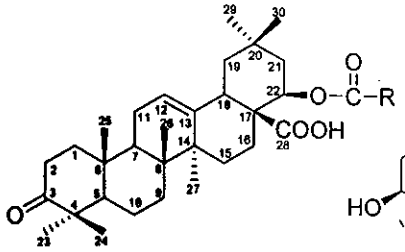
CHAPTER -2

Review of Literature

2.1 Triterpenoids of lantana

Lantana consists of various natural products but triterpenoids attracted the most attention of scientific world because of their toxicity. Most of the triterpenoids isolated from the leaves of *L. camara* are pentacyclic and belong to the oleane series, a few belong to the ursane and lupane series, and some have an oxide bridge from C-3 to C-25. Lantadene A (LA, **1**), Lantadene B (LB, **2**), lantadene C (LC, **3**), and lantadene D (LD, **4**) are the major constituents of *L. camara* (red flower variety) leaves. [7-10] Out of these Lantadene A (22β-angeloyloxy-3-oxoolean-12-en-28-oic acid) is the most abundant triterpenoid of lantana (0.7% dry weight) and derived biosynthetically by the cyclization of squalene. [11] LA and LB are the major constituents of the common pink-edged red flower variety. [12] LA and LB could not be detected in *L. camara* common pink and *L. tiliaefolia.* [12, 13] The structures assigned to the various triterpene metabolites were determined by classical methods and chemical correlation. Furthermore, X-ray crystallographic studies supported the structures assigned to lantadene A [14, 15], B [16] and C. [16] A method for the TLC separation of lantadene A-D [17] and an HPLC method for the quantification of the lantadenes have also been reported. [18] Molecular structures of LA (**1**), LB (**2**), LC (**3**), RLA (**6**), and RLB (**7**) have been determined in 1996. [15, 16, 19] The substitution at the 22 position is *β*-axial and the rings A, B, C, D, and E (Figure 1) are *trans*, *trans*, *trans*, and *cis* fused, forming an extended structure in all the molecules. LA, LB, and LC are similar except for the side chain (at C-22); atoms C-32 and C-33 are connected by a single bond in LC and double bond in LA (Figure 1). Hence, the side chain conformation in LA and LB is identical with that of LC. [15, 16] The differences observed in the side chain conformation of LA, LB, and LC is due to the presence or absence of double bond at C-32. LC has an asymmetric carbon at C-32, but it is absent in lantadene A and B due to the presence of the double bond. The differences in side chain conformation suggest that LC might be binding to a receptor for its bioactivity, where the asymmetric carbon and carbonyl oxygen (O-5) have an important role to play. Further, the presence of two methyl groups at C-33 in LB may be posing steric hindrance to the active site of receptor for hepatotoxicity. It may be speculated that the C-34 atom in LA (form I), which is in *cis* conformation with respect to C-31, may rotate to the *trans* position in form II as in the case of LC, which makes it hepatotoxic. However, the potency of

LA (form II) and LC may differ due to the presence of an asymmetric carbon atom at C-32 in LA. [14-16] Reduced lantadene A (RLA, 6) and reduced lantadene B (RLB, 7) are the minor constituents. [10, 20] Icterogenin (8) has been reported from the leaves and stem of *L. camara* Townswhile pricky orange [12] but found to be absent in *L. camara* red flower variety. [14, 20] Townsville prickly orange has oleanonic acid (10) and ursonic acid (27) as major constituents in its leaves and stems, while LA and LB are only minor constituents. [12] Similarly, LC, RLA, and icterogenin have not been reported in the taxon common pink. [21] This taxon is nontoxic and is commonly grazed upon in New Zealand, where it is most widespread. [22] The profile of triterpenoids in the roots of *L. camara* is different from that in the leaves. Oleanolic acid (5) is the major constituent of the roots of *L. camara* Helidon white, followed by oleanonic acid (10). [12] In the roots of both toxic and non toxic taxa, oleanolic acid is the major constituent. The rootlets and root bark of *L. camara* provides plentiful (2%) supply of oleanolic acid. Roots of *L. indica* yielded an oleane derivative 3β-24-dihydroxyolean-12-en-28-oic acid (9), oleanolic acid (5), 24-formyl-3-oxoolean- 12-en-28-oic acid (34), and ursolic acid (26). [23, 24] Triterpenoids isolated from the roots of Chinese *L. camara* included lantanolic acid (37), 22β-*O*-angeloxylantanolic acid (19), 22β-*O*-senecioyl-oleanolic acid, 22β-hydroxy oleanonic acid, 19α-hydroxy-ursolic acid, and 3β-isovaleroyl-19α-hydroxy-ursolic acid.[25] Lantanolic (37) and lantic acid (14) originally isolated from an Indian sample of *L. camara*, incorporate an oleanane and an ursane skeleton with an unusual hemiketal arrangement between an alcohol at C-25 and the ketone at C-3. [26, 27] Camaric acid (16) and camarinic acid (17) were two new triterpenoids, which were isolated from the aerial parts of *L. camara* were characterized as 22β-acetoxy-3,25 epoxy-3α-hydroxy-12-ursen-28-oic acid and 3,25 epoxy-3α-hydroxy-22β (2-methyl-2Z-butenoyloxy)-12-oleanen-28-oic acid respectively. [28] Another, nematicidal triterpenoid lantanone (35) was isolated from the aerial parts of *L. camara* [29]. Camarolide (11), lancamaric acid (36), ursonic acid (27), uroxy acid (23), methyl ursoxylate (24), ursongillic acid (25), ursethoxy acid (28), camaryolic acid (39), methyl camaralate (40) and camangeloyl acid (41) were also isolated in Pakistan from the aerial parts of *L.camara.* [30] Recently, two new olean-12-ene triterpenoids, camarolic acid (46) and lantrigloylic acid (47), were isolated from the aerial parts of *Lantana camara* and reported their nematicidal activity. [31] Same research group isolated another two new pentacyclic triterpenoids, namely lantanoic acid (48) and camaranoic acid (49) from the aerial parts of *L. camara.* [32]

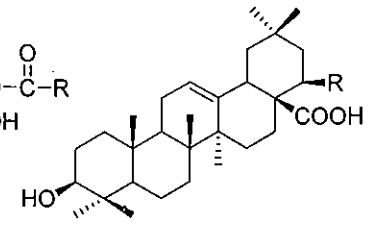


(1) R = -C(CH_3) =CH(CH_3)
Lantadene A

(2) R = -CH=C(CH_3)$_2$
Lantadene B

(3) R = -CH(CH_3)CH_2CH_3
Lantadene C

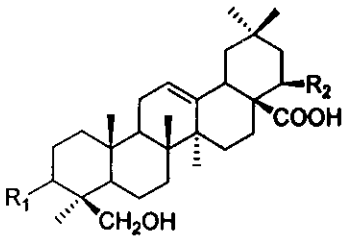
(4) R = -CH(CH_3)$_2$
Lantadene D



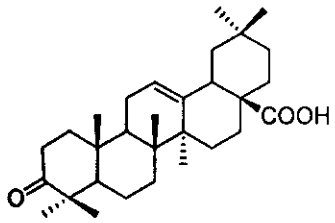
(5) R = H
Oleanolic acid

(6) R = -OCOC(CH_3) =CH(CH_3)
Reduced Lantadene A

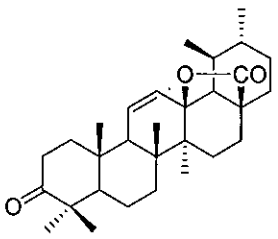
(7) R = -OCO-CH=C(CH_3)$_2$
Reduced Lantadene B



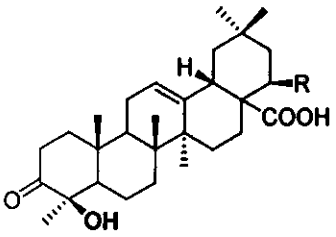
(8) R_1 = O; R_2 = -OCOC(CH_3) =CH(CH_3)
Icterogenin
(9) R_1 = OH; R_2 = H
3ß,24-dihydroxyolean-12-en-28-oic acid



(10) Oleanonic acid

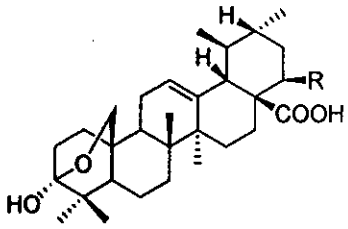


(11) Camarolide

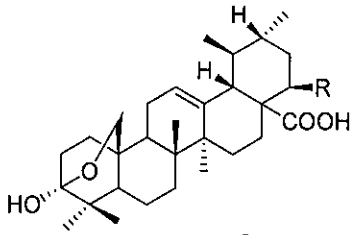


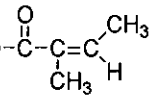
(12) R = H
4- Epihederagonic acid

(13) R = OH
22ß-hydroxy-3-oxolean-12en-28-oic acid

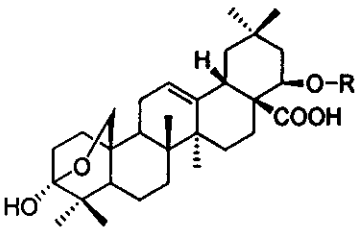


(14) R = H
Lantic acid
(15) R = -OCOCH_3
22ß- acetoxylantic acid



(16) R = 
Camaric acid

(17) R = -OCOCH_3
Camarinic acid



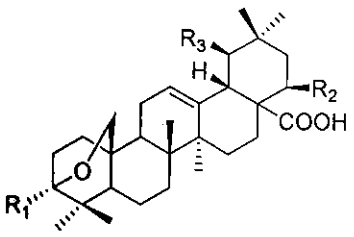
(18) R = -COCH=C(CH_3)$_2$
22ß-Dimethylacryloyloxy lantanolic acid

(19) R = -COC(CH_3)=CH(CH_3)
22ß- Angeloxylantanolic acid

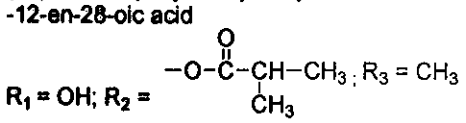
Figure 2: Triterpenoids isolated from *Lantana camara* L.

Another investigation of the methanolic extract of *L. camara* has revealed a suite of euphane triterpene lactones. The presence of these metabolites, which occur in trace quantities 0.00004-0.0002% [33], was detected by using an assay in which thrombin activity was measured as a function of clot formation from fibrinogen. In all, five active principles **(50a-e)** were isolated.

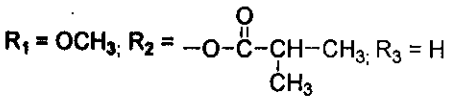
The structure of these compounds was determined by means of spectroscopic methods and confirmed by single crystal X-ray crystallographic studies on **50a**. All compounds were potent inhibitors of human thrombin IC_{50} 18-130 nM and showed comparable activity to hirudin IC_{50} 12 nM, a dried and refined extract of leeches *Hirudo medicinalis.* [33] Structures of major triterpenoids isolated from *lantana* are shown in Figure 1-5.

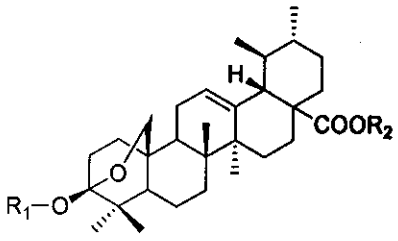


(20) 3ß-25-epoxy-3a-hydroxy-22ß-isobutanoyloxyolean -12-en-28-oic acid

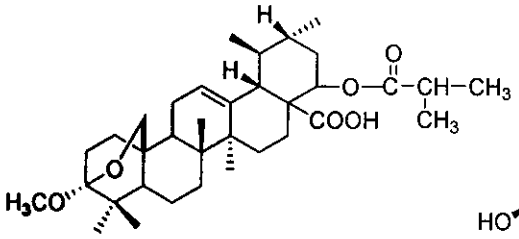


(21) Camarilic acid

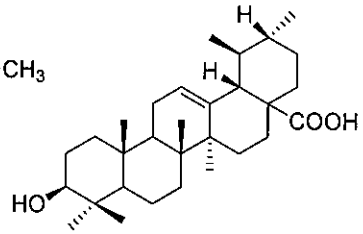




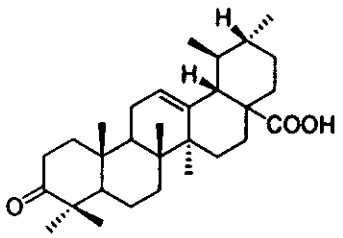
(23) Ursoxy acid	R_1 R_2 CH_3 H
(24) Methyl Ursoxylate	R_1 R_2 CH_3 CH_3
(25) Ursangillic acid	C_2H_5 $-C(CH_3)=CH(CH_3)$



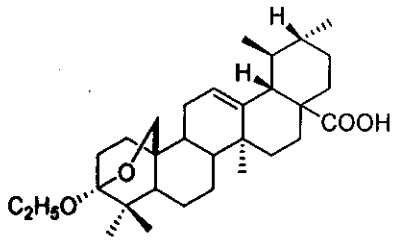
(22) Camaracinic acid



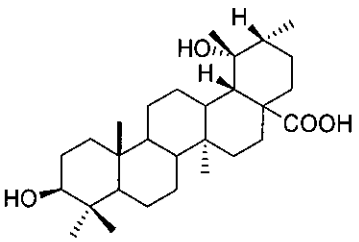
(26) Ursolic acid



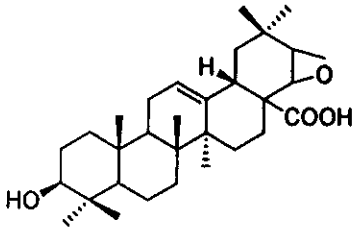
(27) Ursonic acid



(28) Ursethoxy acid

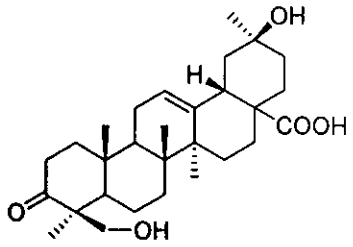


(29) 3-β,19α-Dihydroxy-ursan-28-oic acid

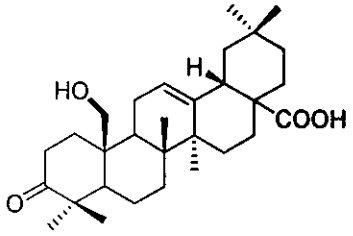


(30) 21,22-Epoxy-3β-hydroxy-olean-28-oic acid

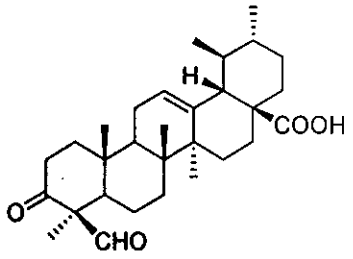
Figure 3: Triterpenoids isolated from *Lantana camara* L. (Cont…)



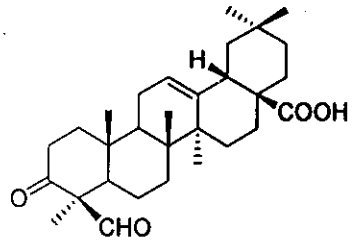
(31) 24-Hydroxy-3-oxours-12-ene-28-oic acid



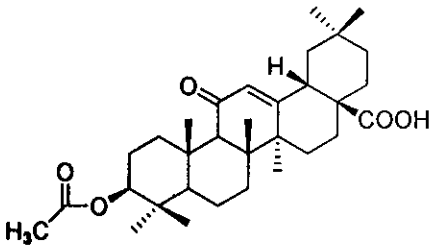
(32) 25-Hydroxy-3-oxoolean-12-ene-28-oic acid



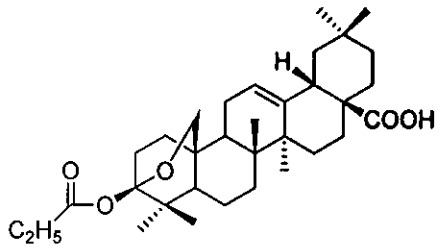
(33) 3,24-Dioxo-urs-12-en-28-oic acid



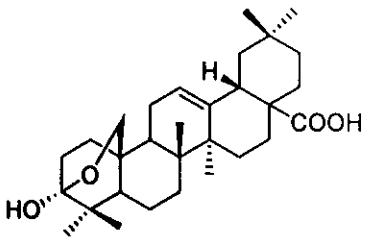
(34) 24-Formyl-3-oxolean-12-en-28-oic acid



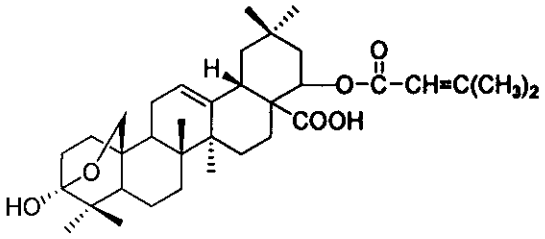
(35) Lantanone



(36) Lancamaric acid

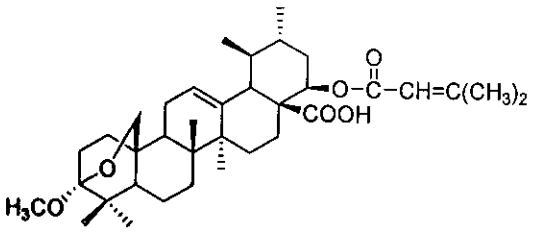


(37) Lantanolic acid

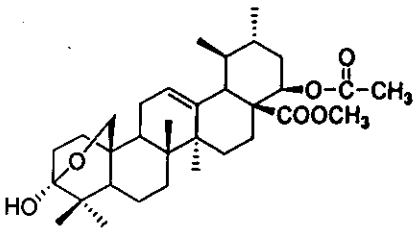


(38) Lantanilic acid

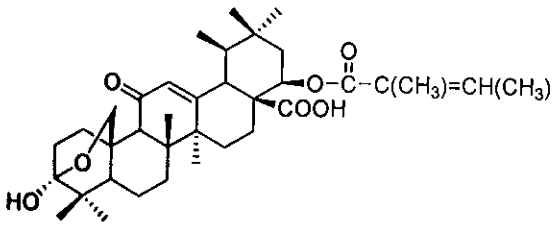
Figure 4: Triterpenoids isolated from *Lantana camara* L. (Cont…)



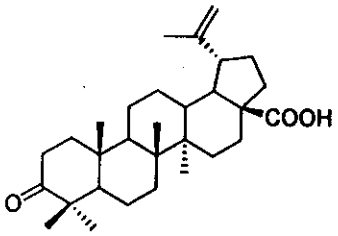
(39) Camaryolic acid



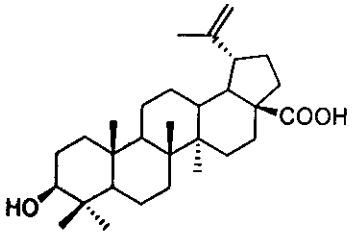
(40) Methyl camaralate



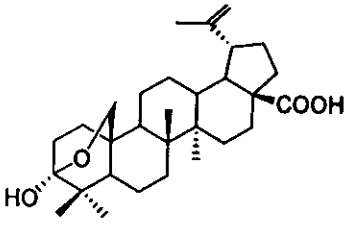
(41) Camangeloyl acid



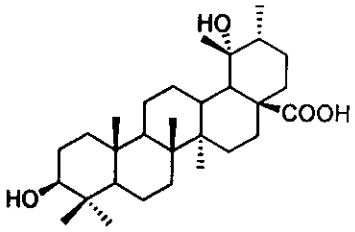
(42) Betulonic acid



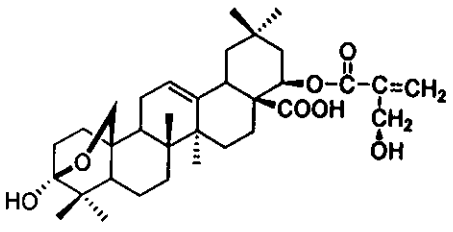
(43) Betulic acid



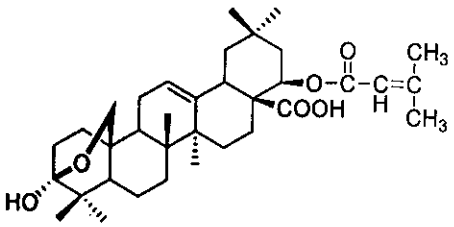
(44) Lantabetulic acid



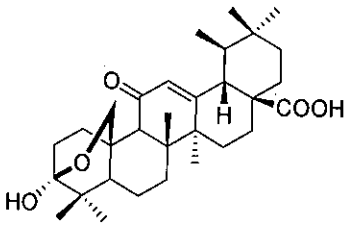
(45) Pomolic acid



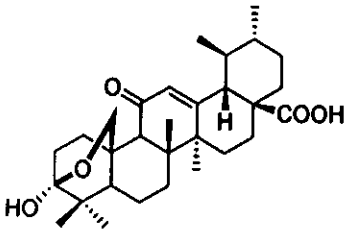
(46) Camarolic acid



(47) Lantrigloylic acid

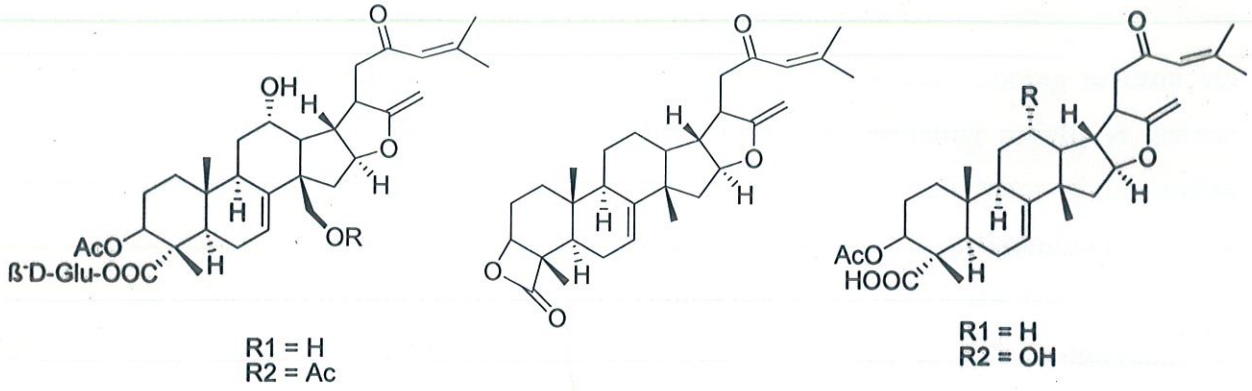


(48) Lantanoic acid



(49) Camaranoic acid

Figure 5: Triterpenoids isolated from *Lantana camara* L. (Cont…)



(50a-e) 5,5- trans fused cyclic lactone containing euphane triterpenoids

Figure 6: Triterpenoids isolated from *Lantana camara* L. (Cont…)

2.2 Biological Activity of lantana triterpenoids

Apart, from their toxicity, LA, LB and LC were found to inhibit Epstein-Barr virus activation in Raje cells induced by 12-*O*- tetradecanoylphorbol-13 acetate (TPA). [54] LA and LB were even active at 10 mol triterpenoid / 1 mol TPA. LA and LB showed inhibitory effects on two–stage carcinogensis of mouse skin papillomas, using 7, 12- dimethylbenz[a]anthracene as an initiator and TPA as promoter. LB (47µg), dosing before each treatment of TPA, delayed the formation of papillomas on mouse skin, reduced the average number of papillomas – bearing mice (by 15% at 20 weeks) and reduced the average number of papillomas / mouse (50% at 20 weeks). [55] LA significantly inhibited cell proliferation of HL-60 cells and induced cell apoptosis through down regulating Bcl-2 and up regulating Bax expression. The peptidic caspase-3 inhibitors DEVD-CHO (NH_2-Asp-Glu-Val-Asp-CHO, 2µM), increased the viability of HL-60 cells, previously treated with LA. [56] LA showed chemoprevention on two-stage carcinogenesis model in Swiss Albino mice by decreasing expression of AP-1 (c-jun), NFκ B (p65) and p53 expression. [57] Recently, LD showed moderate anticancer activity both in *in vitro* and *in vivo* cancer models. [58] Recently, lot of intrest has been shown in anti-inflammatory activity of triterpenoids. [59] Oleanolic acid and urosolic acid have shown significant anti-inflammatory activity (IC_{50} 2-4, 6µM) as inhibitor of human leucocyte elastase (HLE). This enzyme participates in the destruction of elastin and plays a role in chronic disorders such as pulmonary emphysema, cystic fibrosis, hepatitis and rheumatic arthritis.

Oleanolic acid and urosolic acid also possess inhibitory effects on inflammation and on various stages of tumor development. [60] In recent study, ursolic acid was shown to have COX-2 inhibitory activity with an IC_{50} value of 130µM and COX-2 /COX -1 selectivity ratio of 0.6.

Oleanolic acid showed IC_{50} 295μM and a ratio of 0.8. [61] Euphane lactone triterpenoids were found to have thrombin inhibitory activity, which inhibit the blood- clotting cascade via acylation of the active site Ser 195 residue of thrombin. This acylating activity is generic towards other serine proteases. These lactone triterpenoids are potent inhibitor of α-thrombin and to lesser extent, of α-chymotrypsin and other serine proteases. The α-thrombin is a serine protease that belongs to trypsin family and has a central role in the hemostatic process, where it displays both coagulant and anticoagulant activities. [62, 63] The IC_{50} for a-thrombin, a-chymotrypsin and trypsin was 0.004, 0.07 and 0.07 for **32** and 0.004, 0.01, 0.12 mM for **34**. X-Ray crystallographic studies of the α-thrombin-**32** and a-thrombin-**34** complexes showed the inhibitor in the ring opened form. The hydroxyl group that attacks the seryl ester probably occupies the position normally taken by water during deacylation of peptide substrates. Model compounds incorporating 5,5 *trans*-fused indane lactones have been tested as inhibitors of thrombin. [62, 63] Although, some of these showed significant activity as HLE, chymotrypsin and human α -thrombin inhibitors, they were relatively unstable in plasma. Model compounds containing a lactam had much enhanced plasma stability compared to their lactone counterparts and showed appreciable *in vitro* anticoagulant activity. [64]

2.3 Toxicity of Lantana

Apart from its notorious proliferation and negative impact on the environment, *L. camara* has caused illness and even fatality in cattle, horses and sheep. The first field report of lantana toxicity was from Townsville (Australia) in 1910. The toxicity to ruminants has been reported from Australia, New Zealand, South America, Africa and India. Photosensitization is most prominent clinical sign of poisoning. Photosensitive dermatitis occurs within 1 or 2 days and as the disease progresses, large area of skin become necrotic. Jaundice is prominent within 2-3 days with yellowing of sclera and other mucous membranes. Loss of appetite in poisoned animal occurs within 1 day. The most severely poisoned animal dies within 2 days, but usually death occurs after 1-3 weeks of poisoning. The toxicity of lantana was found to be due to its pentacyclic triterpenoids. All toxic taxa contained LA and LB (80 &120mg/kg) and have been toxic to sheep. The 3β- hydroxyl analogue of LA is also toxic (40mg/kg), but toxicity is not as much as in the case of LA and LB. the amount estimated to be present in toxic dose of lantana leaves is 3mg/kg. All species of lantana are not toxic. It is generally accepted that LA, LB, LD, 3β- analogue of LA and icterogenic acid are responsible for the toxicity in sheep, cattle, goat but horses, rats, neonatal claves and lambs are not susceptible to LA. The oral toxic dose of LA for sheep is 60mg/kg and 1-3mg/kg by i.v route. The toxins absorbed from the rumen and small

intestine are transported to liver by portal blood. They are metabolized in liver and secreted in bile, where they injure bile canalicular membrane thus, inhibit bile secretions.

2.4 Lantana in folk medicine

This plant has been used in many parts of the world to treat wide variety of disorders. In Argentina, the plant decoction is used as intestinal stimulant. A "tea" of lantana leaves is a popular folk remedy in Latin America and Bahamas, taken as diuretic and to relieve stomach aches, cold, fever, hypertension and diarrhea. In Ghana, infusion of whole plant was used for bronchitis and powdered root in milk was given to children for stomach ache. The decoction of roots is used as pectoral antiasthmatic and in the treatment of venereal diseases. The leaves are applied to cuts, ulcers and bruises. A strong decoction is antidote for snake venom. In Asian countries, leaves were used to treat cuts and as vermifuge. It has been claimed that steroids, from the leaves exhibited cardioactive properties. Lantamine, an alkaloid from the stem bark and roots of lantana showed antipyretic and antispasmodic properties comparable to those of quinine. Verbascoside from leaves has antitumor, immunosuppressant and antimicrobial activity.

2.5 Utilization of Lantana

There is a big question can we make a "virtue of necessity" so long as we have to live with lantana plant? In case, the lantana plant or its active constituents could be of economic importance, its abundant growth would be of complementary advantage. Lantana twigs are used as source of fuel for cooking and heating in rural sector from years. However, in view of the strong allelopathic action of different parts of lantana, its slurry is used as manure. Partially decomposed lantana foliage, in combination with cattle dug has been utilized to produce biogas. Lantana fruit contains some growth promoters. Plants of wheat sprayed with aqueous extracts of lantana fruit, showed increased growth characteristics. Lantana straw in conjugation with waste paper has been used in cultivation of mushrooms. Lantana roots contain rubber like material, which is suitable for manufacture of rubber. Lantana seeds have supplementary nutritive value. Pyrolysis upto 40% of lantana biomass yields carbon powder. A petroleum-ether extract of the leaves, at a concentration of as low as 0.015%, has produced 100% mortality of fourth instar larvae of *Culex quinquefasciatus* in 24 hours. There is potential for the development of biopesticides, which may not exhibit harmful effects associated with synthetic chemicals. The crude extract of bark of lantana showed juvenilizing effects on the nymphs of *Dysdercus koeniigii* (the cotton stainer). Now days, furniture and cardboard are also developed from wood of lantana. The extract of leaves of lantana exhibited insecticidal activity against

Aphis gossypii, a pest of brinjal and against *Bagrada cruciferarum,* a serious pest of crucifers. The bark of stem and roots of lantana contains quinine like alkaloid with strong antipyretic and antispasmodic activity. Lantana plant is also considered vulnerary, diaphoretic and carminative. The active principals of lantana which are responsible for their biological activity need a serious attention. There is great potential for utilization of lantana plant for various purposes. However, except for routine use of lantana twigs for fuel purposes, none other has been exploited for large scale industrial applications.

CHAPTER -3

Research Envisaged

As discussed in pervious section, weed *Lantana camara* is rich source of chemical compounds with simple to complex structures. The Lantadene A and B are pentacyclic triterpenoid of oleanane series and have shown wide spectrum of biological activities in recent years. Recently, in our lab we have found that C-3 aromatic ester of Lantadene A and B showed remarkable antimicrobial activity against *E. coli* and *Pseudomonas aeruginosa.* Therefore, we decided to synthesize various C-3 aromatic esters of Lantadene A and B and to evaluate them for their antimicrobial activities.

CHAPTER -4

Material and methods

The purity of all compounds was established by single spot on the Merck percoated silica gel TLC plates. Iodine vapor was used for detection. The solvent system used was Hexane (60-70 °C): ethyl acetate (5:1). Melting points were determined on an Indosati digital melting point apparatus and were uncorrected. IR spectra were recorded on a Perkin Elmer-spectrum RX-IFTIR, using potassium bromide pellets. ^{1}H NMR and ^{13}C NMR spectra were recorded with Bruker AVANCE II 400 MHz spectrometer using $CDCl_3$ as solvent, and tetramethylsilane was used as internal standard. Mass spectra were obtained with Micromass 70-VSE mass spectrometer at 70 eV using electronionization (EI). Elemental analysis of compounds was within ± 0.04% of the theoretical values. All solvents were freshly distilled and dried prior to use according to standard procedures.

4.1 Plant collection

Leaves of *L. camara* were collected in the month of August 2012 from Palampur and Waknaghat (HP), India. The leaves were shade-dried and powdered.

4.2 Extraction and isolation of partially purified lantadenes

To 100 g of lantana leaf powder, 500ml methanol was added and incubated for 24h with intermittent shaking. The extract was separated by filtration through a muslin cloth and decolorized with 20 g of activated charcoal, which yielded a golden yellow extract. The solvent was removed under reduced pressure; the residue was suspended in a methanol-water (1:7) mixture and extracted with chloroform ($CHCl_3$, 2 × 15 ml). The organic layer was dried over anhydrous sodium sulphate and the solvent was removed under reduced pressure. The solid residue obtained was recrystallized from methanol to obtain partially purified lantadenes (1.06 g, 1.06%) as a white crystalline product.

4.3 Purification of lantadene A by recrystallization

1 kg of lantana leaves powder was extracted with 5lt ethyl acetate at R.T. for 24 hr with intermittent shaking. The extract was filtered and 250 gm of activated charcoal was added to it and kept for 1 hr. The filtrate was concentrated under reduced pressure and dissolved in 100 ml chloroform and partitioned with 100 ml water. The aqueous layer was washed again with

chloroform (100 ml×2). The organic layer was evaporated in rota evaporator to give mixture of lantadenes (4.48±0.216 gm). The 1 gm mixture of lantadenes were taken in 200 ml solvent of methanol-THF (190 ml+10 ml) and heated to boiling and kept in freeze at 4 °C till precipitation appeared and residue obtained after filtration was stored as lantadene A. Same procedure was repeated twice for remaining filtrate and residues obtained in three attempts were pooled to give 361 mg of total lantadene A with partial impurity of lantadene B, while most of the lantadene B remained in the filtrate. The purified lantadene A was again recrystallized twice to give 204 mg pure lantadene A (20.4% w/w).

4.4 Purification of lantadene B by recrystallization

1 kg of lantana leaves powder was extracted with 5lt ethyl acetate at R.T. for 24 hr with intermittent shaking. The extract was filtered and 250gm of activated charcoal was added to it and kept for 1 hr and filtered again. The filtrate was concentrated under reduced pressure and dissolved in 100 ml chloroform and partitioned with 100 ml water. The aqueous layer was washed again with chloroform (100 ml×2). The organic layer was evaporated in rota evaporator to give a mixture of lantadenes (4.48±0.216 gm). The 500mg mixture of lantadenes were taken in 100 ml solvent comprised of methanol-THF (95 ml+5ml) and heated to boiling and kept in freeze at 4 °C till crystals appeared. The crystals obtained were separated as lantadene A while lantadene B remained in filtrate. Same procedure of recrystallization was repeated further three times for filtrate obtained on each time. The filtrate obtained in last step (4th attempt) was again subjected for recrystallization to give 8.13 mg pure lantadene B (1.62% w/w).

22β-[(2-methyl-1-oxo-2-butenyl)oxy]-3-oxoolean-12-en-28-oic acid (1)

White solid (yield 52%); mp 285-286 °C; IR (KBr) ν'max 2952.45 (C–H stretching), 1715.85 (C=O, ester), 1702.14 (C=O, 3-keto); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.82 (3H, s, CH_3), 0.85 (3H, s, CH_3), 1.00 (3H, s, CH_3), 1.05 (6H, s, 2 x CH_3), 1.09 (3H, s, CH_3), 1.17 (3H, s, CH_3), 3.05 (1H, d, J = 10.40 Hz, C-18-H), 5.09 (1H, s, C-22-H), 5.38 (1H, s, C-12-H), 6.00 (1H, dd, J = 7.04; 7.12 Hz, C-3′-H); ^{13} C NMR (100 MHz, $CDCl_3$): 37.72 (C-1), 34.14 (C-2), 217.72 (C-3), 38.45 (C-4), 55.29 (C-5), 21.48 (C-6), 30.19 (C-7), 39.21 (C-8), 50.59 (C-9), 36.78 (C-10), 24.19 (C-11), 122.49 (C-12), 143.10 (C-13), 45.94 (C-14), 26.44 (C-15), 23.51 (C-16), 46.88 (C-17), 41.99 (C-18), 47.45 (C-19), 30.05 (C-20), 33.69 (C-21), 75.84 (C-22), 27.56 (C-23), 16.84 (C-24), 15.67 (C-25), 19.48 (C-26), 26.14 (C-27), 179.28 (C-28), 32.19 (C-29), 25.79 (C-30), 166.26 (C-1'), 127.58 (C-2'), 139.06 (C-3'), 15.10 (C-4'), 20.58 (C-5'); ESI-MS (*m/z*): 553.4 [M+1]; *anal.* $C_{35}H_{52}O_5$ (552.5): C, 76.05%; H, 9.48%; found C, 76.03%; H, 9.50%.

22β-[(3-methyl-1-oxo-2-butenyl)oxy]-3-hydroxyolean-12-en-28-oic acid (2)

White solid (yield 39%); mp 283-284 °C; IR (KBr) ν max 2952.45 (C–H stretching), 1715.85 (C=O, ester), 1702.14 (C=O, 3-keto); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.83(3H, s, CH_3), 0.88 (3H, s, CH_3), 1.00 (3H, s, CH_3), 1.05 (6H, s, 2 x CH_3), 1.09 (3H, s, CH_3), 1.17 (3H, s, CH_3), 3.02 (1H, d, J = 9.96 Hz, C-18-H), 5.04 (1H, s, C-22-H), 5.37 (1H, s, C-12-H), 5.55 (1H, s, C-2′-H); ^{13} C NMR (100 MHz, $CDCl_3$): 38.54 (C-1), 33.75 (C-2), 217.81 (C-3), 39.16 (C-4), 55.30 (C-5), 21.50 (C-6), 32.26 (C-7), 39.24 (C-8), 50.57 (C-9), 37.63 (C-10), 25.77 (C-11), 122.37 (C-12), 143.09 (C-13), 45.97 (C-14), 27.46 (C-15), 24.13 (C-16), 46.87 (C-17), 42.07 (C-18), 47.45 (C-19), 30.07 (C-20), 36.77 (C-21), 75.20 (C-22), 27.59 (C-23), 16.85 (C-24), 15.16 (C-25), 19.52 (C-26), 26.44 (C-27), 178.84 (C-28), 34.16 (C-29), 26.28 (C-30), 165.32 (C-1'), 115.96 (C-2'), 157.15 (C-3'), 20.25 (C-4'), 23.56 (C-5'); ESI-MS (*m/z*): 553.5 [M+1]; *anal.* $C_{35}H_{52}O_5$ (552.5): C, 76.05%; H, 9.48%; found C, 76.07%; H, 9.49%.

4.5 Reduction of Lantadene A and B

The sequence of steps involved in conversion of Lantadene A & B to reduced Lantadene A & B is shown in Scheme 1. To 100 mg (0.18 mM) of **1** and **2** and 6.80 mg (0.18 mM) of sodium borohydride were separately subjected for microwaves irradiations at 210W (30%) for 1 to 4 minutes in a 2 ml solution of methanol (1ml) and tetrahydrofuran (1ml). The reaction was monitored on TLC after every 1 minute and was incomplete up to three minutes and at 4 minutes reaction was completed to afford **3** and **4** respectively. After completion of reactions dilute HCl solution was added to quench the unused $NaBH_4$. The organic solvents were evaporated in rota evaporator and precipitated reduced lantadenes were extracted with dichloromethane (DCM). The solvent was removed under reduced pressure to give **3** (TLC, petroleum ether: ethyl acetate; 4:1, R_f 0.31) and **4**, (TLC, petroleum ether: ethyl acetate; 4:1, R_f 0.28). The product was recrystallized from methanol.

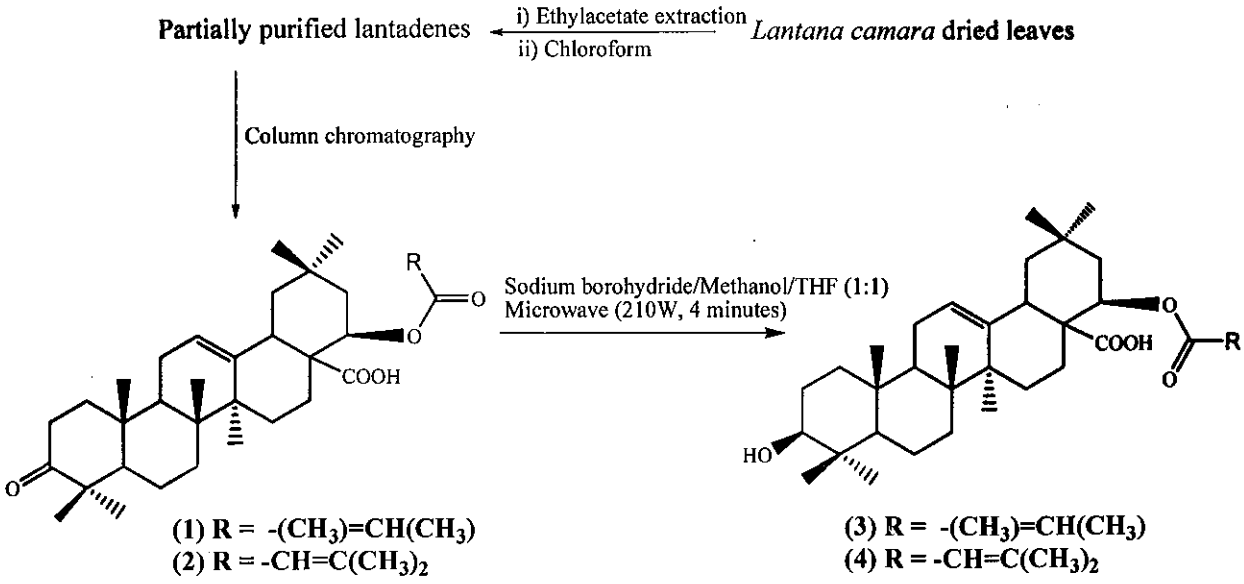
22β-[(3-Methyl-1-oxo-2-butenyl) oxy]-3β-hydroxyolean-12-en-28-oic acid (3)

White crystals (yield 100.6 mg, 99.70% w/w), mp 278-279 °C, IR (ν_{max}, KBr, cm^{-1}): 3482.87 (3-OH stretch), 2948.99, 2827.53 (C–H stretching), 1717.87 (ester, C=O), 1701.25 (acid, C=O), ^{1}H NMR (DMSO-*d6*, δ ppm), 6.00 (1H, *m,* C-3′-H), 5.31 (1H, *t,* C-12-H), 4.99 (1H, *t,* C-22-H), 3.09 (1H, *t, J=7.82*, C-3-H), 3.00 (1H, *d,* C-18-H), 1.16 (3H, *s,* CH_3), 1.09 (3H, *s,* CH_3), 1.06 (3H, *s,* CH_3), 0.99 (3H, *s,* CH_3), 0.85 (3H, *s,* CH_3), 0.83 (3H, *s,* CH_3). ^{13} C NMR ($CDCl_3$, δ ppm): 38.77 (C-1), 36.51 (C-2), 79.28 (C-3), 45.71 (C-4), 54.77 (C-5), 20.11 (C-6), 32.30 (C-7), 40.25 (C-8), 47.06 (C-9), 38.09 (C-10), 23.74 (C-11), 121.53 (C-12), 158.43 (C-13), 41.46 (C-14), 27.90 (C-15), 25.40 (C-16), 49.59 (C-17), 38.99 (C-18), 43.96 (C-19), 29.59 (C-20),

38.29 (C-21), 75.60 (C- 22), 26.96 (C-23), 15.57 (C-24), 15.18 (C-25), 16.49 (C-26), 26.68 (C-27), 177.14 (C-28), 33.43 (C-29), 25.77 (C-30), 165.70 (C-1′), 127.50 (C-2′), 137.42 (C-3′), 15.00 (C-4′), 22.89 (C-5′). ESI-MS (*m/z*): 555.5 [M+1], *anal.* $C_{35}H_{54}O_5$, C, 75.77%, H, 9.81%, found C, 75.75%, H, 9.80%.

22β-[(3-Methyl-1-oxo-2-butenyl) oxy]-3β-hydroxyolean-12-en-28-oic acid (4)

White crystals (yield 99.26 mg, 98.90% w/w), mp 276-278 °C., IR (ν_{max}, KBr, cm^{-1}): 3480.79 (3-OH stretch), 2949.59, 2875.08 (C–H stretching), 1717.98 (ester, C=O), 1701.98 (acid, C=O), ^{1}H NMR ($CDCl_3$, δ ppm): 5.51 (1H, *d, J= 2.76,* C-2′-H), 5.31 (1H, *t,* C 12-H), 4.98 (1H, *t,* C-22-H), 3.15 (1H, *q, J= 4.56,* C-3-H), 2.95 (1H, *d,* C-18-H), 1.13 (3H, *s,* CH_3), 1.10 (3H, *s,* CH_3), 1.06 (3H, *s,* CH_3), 1.00 (3H, *s,* CH_3), 0.88 (3H, *s,* CH_3), 0.85(3H, *s,* CH_3), ^{13}C NMR ($CDCl_3$, δ ppm): 38.49 (C-1), 33.81 (C-2), 79.02 (C-3), 47.64 (C-4), 55.20 (C-5), 20.24 (C-6), 30.08 (C-7), 39.25 (C-8), 48.55 (C-9), 35.37 (C-10), 23.85 (C-11), 122.68 (C-12), 143.09 (C-13), 41.95 (C-14), 28.11 (C-15), 24.17 (C-16), 50.55 (C-17), 38.76 (C-18), 46.05 (C-19), 29.71 (C-20), 37.05 (C-21), 75.24 (C-22), 27.63 (C-23), 15.58 (C-24), 15.45 (C-25), 16.95 (C-26), 26.30 (C-27), 177.58 (C-28), 31.15 (C-29), 25.87 (C-30), 165.42 (C-1′), 116.05 (C-2′), 157.16 (C-3′), 23.51 (C-4′), 27.45 (C-5′). ESI-MS (*m/z*): 555.4 [M+1], *anal.* $C_{35}H_{54}O_5$, C, 75.77%, H, 9.81%, found C, 75.72%, H, 9.82.



Scheme 1: Isolation of Lantadene and their reduction

4.6 Preparation of 22β-hydroxy-3-oxoolean-12-en-28-oic acid (5)

To a solution of lantadenes A and B (100 mg, 0.18 mM) in ethanolic potassium hydroxide solution (10% w/v, 25 mL) was added and the reaction mixture was refluxed for 6 hr. After reaction completion, the solvent was removed *in vacuo* and the residue was diluted with water (15mL). The mixture was acidified with dilute HCl and extracted with ethyl acetate (3 x 15 mL). The combined organic layer was dried over anhydrous sodium sulfate and evaporated to dryness. The residue was chromatographed over silica gel (100-200 mesh) and eluted with ethyl acetate: n-hexane (1:4) to obtain **5**.

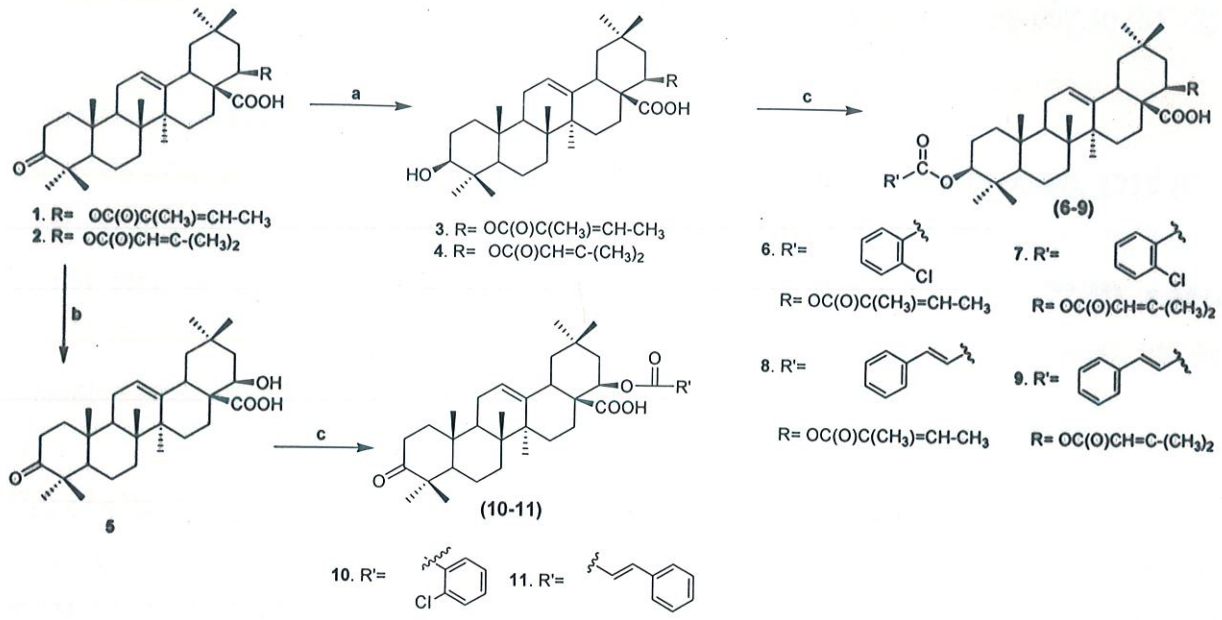
White solid (0.054g, 63.9%); mp, 234-236 ^{0}C; IR (KBr) ν'max (cm^{-1}): 3434 (O-H stretch), 2946 (C-H stretching), 1703 (C=O, ketone); ^{1}H NMR (400 MHz, $CDCl_3$): 0.78 (3H, s, CH_3), 0.83 (3H, s, CH_3), 0.97 (3H, s, CH_3), 0.99 (3H, s, CH_3), 1.02 (3H, s, CH_3), 1.05 (3H, s, CH_3), 1.09 (3H, s, CH_3), 2.28-2.33 (1H, m, C-2a-H), 2.44-2.51 (1H, m, C-2b-H), 2.94 (1H, dd, J = 4.08; 4.12 Hz, C-18-H), 3.85 (1H, t, J = 3.24 Hz, C-22-H), 5.29 (1H, t, J = 3.44 Hz, C-12-H); ^{13}C NMR (100 MHz, $CDCl_3$): 39.28 (C-1), 34.16 (C-2), 217.84 (C-3), 47.44 (C-4), 55.30 (C-5), 19.54 (C-6), 32.22 (C-7), 39.15 (C-8), 46.90 (C-9), 36.79 (C-10), 24.32 (C-11), 122.41 (C-12), 143.28 (C-13), 42.13 (C-14), 27.80 (C-15), 23.56 (C-16), 52.32 (C-17), 41.23 (C-18), 46.02 (C-19), 30.15 (C-20), 38.00 (C-21), 74.34 (C-22), 26.48 (C-23), 21.48 (C-24), 15.13 (C-25), 16.93 (C-26), 25.75 (C-27), 180.70 (C-28), 33.88 (C-29), 27.16 (C-30); ESI-MS (*m/z*): 469 (M-1); *anal.* $C_{30}H_{46}O_4$ (470.68): C 76.55%, H 9.85%; found: C 76.55%, H 9.84%.

4.7 Synthesis of 3β-substituted and 22β-substituted olean-12-en-28-oic acids (6–11) (Scheme 2)

Compounds **6-11,** were synthesized in two steps. In the first step, acidic group of aldehydes was converted into anhydride group. Equimolar quantity of appropriate aldehyde and acetyl chloride in the presence of pyridine were refluxed in dichloromethane for 4–5 h. Reaction mixture was concentrated and washed with chloroform (100 mL×3) under reduced pressure at 60–65 °C to afford solid to semisolid anhydride products of respective aldehyde, which were used in the next step without further purification.

In the second step, equimolar amounts of compound and appropriate anhydride was refluxed in pyridine in the presence of 4-DMAP for 10–14 h at 92–95 °C. Reaction mixture was poured into 10% HCl solution and precipitated product was extracted with dichloromethane and washed further three times with 10% HCl solution (100 mL×3). Organic layer was evaporated to dryness and the reaction mixture obtained was chromatographed over silica gel (100-200 mesh) and eluted with varying ratio of hexane-ethyl acetate to give purified products (**6–11**).

Scheme 2. Synthesis of lantadene hybrid compounds 6–11[a]



[a]Reagents and conditions: (a) $NaBH_4$, MeOH-THF, stir 7 h; (b) 10% Ethanolic KOH, reflux 6 h; (c) R′-CO-O-CO-CH_3, 4-DMAP, pyridine, reflux 92–95 °C, 10–14 h.

(3*β*)-(2-Chlorobenzoyloxy)-22*β*-angeloyloxy-olean-12-en-28-oic acid (6)

Yield: 54.20%. Mp: 177-175 °C. IR (KBr, cm^{-1}): 3455 (O-H), 2948, 2877 (C-H), 1721 (C=O ester), 1651, 1593 (C=C). ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 7.2717-7.9691 (4H, m, Ar-H), 6.0081-5.9508 (1H, m, C-33-H), 5.3634 (1H, s, C-12-H), 5.0753-5.0825 (1H, t, C-22-H), 4.4826-4.5222 (1H, t, J = 15.84 Hz, C-3-H), 3.0253-3.0595 (1H, dd, J = 5.28, 9.96 Hz, C-18-H). ESI-MS (negative-ion mode, *m/z*): 691.60 (M^-).

(3*β*)-(2-Chlorobenzoyloxy)-22*β*-senecioyloxy-olean-12-en-28-oic acid (7)

Yield: 52.43%. Mp: 172-173 °C. IR (KBr, cm^{-1}): 2949, 2877 (C-H), 1717 (C=O ester), 1651, 1593 (C=C). ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 7.3123-7.8189 (4H, m, Ar-H), 5.5647-5.5740 (1H, m, C-32-H), 5.3715-5.3905 (1H, t, J = 7.60 Hz, C-12-H), 5.0433-5.0583 (1H, t, J = 6.00 Hz, C-22-H), 4.4810-4.5199 (1H, t, J = 15.56 Hz, C-3-H), 3.0122-3.0585 (1H, dd, J = 4.88, 13.76 Hz, C-18-H). ESI-MS (negative-ion mode, *m/z*): 691.60 (M^-).

22*β*-(2-Chlorobenzoyloxy)-3-oxo-olean-12-en-28-oic acid (8)

Yield: 44.77%. Mp: 147-148 °C. IR (KBr, cm^{-1}): 2947, 2875 (C-H), 1745, 1715 (C=O ester), 1635 (C=C). ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 7.0399-7.9101 (4H, m, Ar-H), 5.3250-

5.3338 (1H, t, J = 3.52 Hz, C-12-H), 5.2060-5.2206 (1H, t, J = 5.84 Hz, C-22-H), 3.0251-3.0594 (1H, t, J = 5.25, 13.20 Hz, C-18-H). ESI-MS (negative-ion mode, *m/z*): 607.50 (M^-–2).

(3*β*)-(Cinnamoyloxy)-22*β*-angeloyloxy-olean-12-en-28-oic acid (9)

Yield: 66.07%. Mp: 188-189 °C. IR (KBr, cm^{-1}): 3266 (O-H), 2950, 2877 (C-H), 1719 (C=O ester), 1649 (C=C). ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 7.2634-7.6886 (5H, m, Ar-H), 5.9596-6.0151 (1H, m, C-33-H), 5.3582 (1H, s, C-12-H), 5.0354-5.0829 (1H, t, C-22-H), 4.4810-4.5203 (1H, t, J = 15.72 Hz, C-3-H), 3.0267-3.0610 (1H, t, J = 13.72 Hz, C-18-H). ESI-MS (negative-ion mode, *m/z*): 683.70 (M^-–1).

(3*β*)-(2-Cinnamoyloxy)-22*β*-senecioyloxy-olean-12-en-28-oic acid (10)

Yield: 65.45%. Mp: 185-186 °C. IR (KBr, cm^{-1}): 3240 (O-H), 2947, 2875 (C-H), 1745, 1715 (C=O ester), 1635 (C=C). ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 7.2654-7.6887 (5H, m, Ar-H), 5.5490-5.5552 (1H, t, J = 2.48 Hz, C-33-H), 5.3506-5.3601 (1H, t, J = 3.80 Hz, C-12-H), 5.0350-5.0808 (1H, t, C-22-H), 4.6323-4.6722 (1H, t, J = 15.96 Hz, C-3-H), 3.0231-3.0540 (1H, dd, J = 5.22, 13.37 Hz, C-18-H). ESI-MS (negative-ion mode, *m/z*): 683.70 (M^-–1).

22*β*-(2-Cinnamoyloxy)-3-oxo-olean-12-en-28-oic acid (11)

Yield: 48.90%. Mp: 155-156 °C. IR (KBr, cm^{-1}): 2950, 2872 (C-H), 1738, 1699 (C=O ester), 1632 (C=C). ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 7.2634-7.6769 (5H, m, Ar-H), 5.4013-5.4195 (1H, t, J = 7.28 Hz, C-12-H), 5.0952-5.1113 (1H, t, J = 6.44 Hz, C-22-H), 3.1008-3.1467 (1H, t, J = 4.40, 13.88 Hz, C-18-H). ESI-MS (negative-ion mode, *m/z*): 599.60 (M^-–1).

4.8. In vitro antibacterial activity

Antimicrobial susceptibility testing was carried out by micro dilution broth assay. Briefly, the inoculums were prepared from mid-logarithmic phase bacterial cultures. Each well of 96-well polypropylene microtiter plate (SIGMA) was inoculated with 90 µL of approximately 10^5 CFU/mL of bacterial suspension per mL of Mueller-Hinton broth (HIMEDIA). Then 10 µL of serially diluted compound in 0.04% DMSO over concentration ranging from 0.7-100 µg/mL was added to the wells of microtiter plate. The microtiter plates were incubated overnight with agitation at 37 °C and absorbance was read at 600 nm after 18 h. Cultures (approximately 10^5 CFU/ mL) without compound were used as positive control. Uninoculated Mueller-Hinton

broth was used as negative control. The tests were carried out in triplicate. The minimum inhibitory concentration (MIC) is defined as the lowest concentration of compound that completely inhibits growth.

CHAPTER -5

Results and Discussion

The lantana leaves were dried in the shade and powdered. Lantana leaf powder was extracted with methanol and the extract obtained was treated with charcoal to remove the green pigments which gave golden yellow colored extract. The solvent was removed under reduced pressure and the residue was suspended in methanol-water (1:7) mixture and extracted with chloroform. The organic layer was dried over anhydrous sodium sulphate and the solvent was removed under reduced pressure. The solid residue obtained was recrystallized from methanol to obtain partially purified lantadenes as a white crystalline product. Partially purified lantadenes fraction was chromatographed on silica gel G column 100-200 mesh) using hexane-ethyl acetate (4:1) as the eluting solvent to obtain LA (1) and LB (2). The ESI-MS spectrum of 1 showed peak at m/z 551.3 (M-1) corresponding to the molecular formula $C_{35}H_{52}O_5$. Presence of keto and acid functionality was indicated by IR spectrum absorption bands at 1736.06 (C=O, keto) and 1702.14 cm-1 (C=O, acid). Seven tertiary methyl singlets (δH 1.17, 1.09, 1.05 x 2, 1.00, 0.85 and 0.82), δH 2.35-2.38 (m, 1H, C-2a-H), 2.51-2.60 (m, 1H, C-2b-H), 3.05 (d, J = 10.40 Hz, 1H, C-18-H), 5.38 (s, 1H, C-12-H) in the ^{1}H NMR spectrum and δC 122.49 (C-12), 143.10 (C-13), 179.29 (C-28), 217.72 (C-3) in the ^{13}C NMR spectrum revealed that it belongs to the oleanane series (pentacyclic triterpenoic acid). Presence of ester linkage with angeloyloxy group was characterized by absorption band at 1715.85 cm^{-1} (C=O, ester) in IR spectrum. The ^{1}H NMR showed a singlet at δ 5.09 for C-22α-H and quartet at δH 6.00 for C-3'-H. Similarly in ^{13}C NMR, the C-1', C-2' and C-3' were observed at δC 166.27, 127.59, 139.07 respectively. The presence of peak at m/z 469.3 by loss of $CH_3CH{=}CCH_3COOH$ confirmed the structure.

The ESI-MS spectrum of 2 showed peak at m/z 551.3 (M-1) corresponding to the molecular formula $C_{35}H_{52}O_5$. Presence of keto and acid functionality was indicated by IR spectrum absorption bands at 1738.61 (C=O, keto) and 1692.62 cm-1 (C=O, acid). Seven tertiary methyl singlets (δH 1.17, 1.09, 1.05 x 2, 1.00, 0.85 and 0.82), δH 2.35-2.39 (m, 1H, C-2a-H), 2.51-2.60 (m, 1H, C-2b-H), 3.02 (d, J = 9.96 Hz, 1H, C-18-H), 5.38 (s, 1H, C-12-H) in the ^{1}H NMR spectrum and δ C 122.37 (C-12), 143.09 (C-13), 178.84 (C-28), 217.81 (C-3) in the ^{13}C NMR spectrum revealed that it belongs to the oleanane series (pentacyclic triterpenoic acid). Presence of ester linkage with dimethylacryloyloxy group was characterized by absorption band at 1712.29 cm^{-1} (C=O, ester) in IR spectrum. The ^{1}H NMR showed a singlet at δH 5.04 for C-22α-H and singlet at δH 5.55 for C-2'-H. Similarly in ^{13}C NMR, the C-1', C-2' and C-3' were

observed at δC 165.32, 115.96, 165.32 respectively. The presence of peak at m/z 469.3 by loss of $(CH_3)_2C$=CHCOOH confirmed the structure.

One step reduction of LA and LB with $NaBH_4$ in CH_3OH: THF mixture (1:1) was done by stirring at room temperature to obtain corresponding reduced lantadene A (3) (RLA) and reduced lantadene B (4) (RLB), respectively. The ESI-MS spectrum of 3 showed peak at m/z 553.4 (M-1) corresponding to the molecular formula $C_{35}H_{54}O_5$. Absence of 1736.06 cm^{-1} (C=O, keto) and δc 217.72 (C=O, C-3) while presence of δH 3.09 (t, J = 7.24 Hz, 1H, C-3α-H) and δC 79.12 (C-3β-OH) confirmed reduction process. Presence of 1717.87 cm^{-1} (ester, C=O) in IR spectrum, δH 4.99 (s, 1H, C-22α-H), 6.00 (q, J = 6.08 Hz, C-3'-H) in ^{1}H NMR spectrum, and δC 166.32 (C-1'), 127.68 (C-2'), 138.88 (C-3') in ^{13}C NMR spectrum indicated that $NaBH_4$ did not show any influence on C-22 angeloyloxy ester linkage and Δ2' olefinic bond of LA. It was further confirmed by the presence of peak at m/z 471.3 by loss of CH_3CH=CCH_3COOH. The ESI-MS spectrum of 4 showed peak at m/z 553.4 (M-1) corresponding to the molecular formula $C_{35}H_{54}O_5$. Absence of 1738.61 cm-1 (C=O, keto) and δC 217.81 (C=O, C-3) while presence of δH 3.15 (dd, J = 10.12, 2.96 Hz, 1H, C-3α-H) and δC 79.05 (C-3, Cβ-OH) confirmed the reduction process. Presence of 1717.98 cm-1 (C=O, ester) in IR spectrum, δH 4.96 (s, 1H, C-22α-H), 5.48 (s, 1H, C-3'-H) in ^{1}H NMR spectrum and δC 165.17 (C-1'), 115.99 (C-2'), 152.24 (C-3') in ^{13}C NMR spectrum indicated that $NaBH_4$ did not show any influence on C-22 dimethylacryloyloxy ester linkage and Δ2' olefinic bond of LB. It was confirmed by presence of peak at m/z 471.3 by the loss of $(CH_3)_2C$=CHCOOH.

The 22β-hydroxy-3-oxoolean-12-en-28-oic acid was obtained by the hydrolysis of partially purified lantadenes in ethanolic potassium hydroxide. The ESI-MS spectrum of 1 and 2 showed peak at m/z 469.3 (M-1) corresponding to the molecular formula $C_{30}H_{46}O_4$. It showed IR absorption bands at 3434 (O-H), 1701.98 cm^{-1} (C=O, acid). Absence of characteristic signals of angeloyloxy and dimethylacryloyloxy groups confirmed the cleavage of C-22 ester linkage. Presence of seven tertiary methyl singlets (δH 1.09, 1.05, 1.02, 0.99, 0.97, 0.83 and 0.78), δH 2.28-2.33 (m, 1H, C-2a-H), 2.44-2.51 (m, 1H, C-2b-H), 2.94 (dd, J = 16.72, 6.32 Hz, 1H, C-18-H), 3.85 (t, J = 3.24 Hz, 1H, C-22α-H), 5.29 (t, J = 3.44 Hz, 1H, C-12-H) in ^{1}H NMR spectrum and δC 122.41 (C-12), 143.28 (C-13), 180.70 (C-28) in ^{13}C NMR spectrum indicated that hydrolytic cleavage did not influence oleanane framework.

Compounds **1–6,** were synthesized in two steps. In the first step, acidic group of aldehydes was converted into anhydride group. Equimolar quantity of appropriate aldehyde and acetyl chloride in the presence of pyridine were refluxed in dichloromethane for 4–5 h. Reaction mixture was concentrated and washed with chloroform (100 mL×3) under reduced pressure at 60–65 °C to afford solid to semisolid anhydride products of respective aldehyde, which were used in the next step without further purification.

In the second step, equimolar amounts of compound and appropriate anhydride was refluxed in pyridine in the presence of 4-DMAP for 10–14 h at 92–95 °C. Reaction mixture was poured into 10% HCl solution and precipitated product was extracted with dichloromethane and washed further three times with 10% HCl solution (100 mL×3). Organic layer was evaporated to dryness and the reaction mixture obtained was chromatographed over silica gel (100-200 mesh) and eluted with varying ratio of hexane-ethyl acetate to give purified products **(6–11).** The structures were confirmed by combined use of spectral and elemental analysis.

All the hybrid compounds (**6-11**) were evaluated against Gram positive bacterial strain *S. aureus* and Gram negative bacterial strain *E. Coli*. Results of antibacterial activity are presented in **Table 1.**

Table 1. Antibacterial activity of compounds **6-11**

Compound	MIC (µg/mL)	
	S. aureus (MTCC 3160)	*E. coli (MTCC 723)*
6	12.5	25
7	12.5-25	25-50
8	50	75
9	**6.21-12.5**	**12.5**
10	12.5-25	25
11	50	50

Cinnamic acid-lantadenes hybrid compounds were more active than 2-chlorobenzoic acid counterparts. All the hybrid compounds showed minimum inhibitory concentration (MIC) values between 6.21 and 75 µg/ml against *S. aureus* and *E. Coli*. The most active compound **(9)** showed MIC values of 6.21-12.5 and 12.5 µg/ml against *S. aureus* and *E. Coli*, respectively. Derivatives of lantadene B were less active than the derivatives lantadene A, while derivatives synthesized at C-22 position were found to be least active.

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