STUDIES ON THE ANTIMICROBIAL ACTIVITY OF AVAILABLE TRITERPENOIDS AND ITS DERIVATIVES FROM SOME MEDICINAL PLANTS

THESIS SUBMITTED FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN SCIENCE (BOTANY) UNDER THE UNIVERSITY OF NORTH BENGAL

Thesis Re Submitted by

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Madhumita Chakraborty 27.08.2012

Madhumita Chakraborty

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Abbreviations

Na_2SO_4	=	Sodium sulphate
PtO_2	=	Platinum Oxide
NBS	=	N-Bromo Succinimide
DMSO	=	Dimethylsulphoxide
NaOH	=	Sodium hydroxide
PDA	=	*Potato Dextrose Agar*
Mp	=	Melting point
TLC	=	Thin Layer Chromatography
UV	=	Ultra violet ray
CD	=	Circular dicroism
NMR	=	Nuclear magnetic resonance
IR	=	Infra red
DMF	=	N,N-dimethylformamide
MMP	=	Mixed melting point
$CDCl_3$	=	Deuterited Chloroform
MeOH	=	Methanol

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2. Friedelin
3. Lupeol
4. Betulinic Acid
5. Betulin
6. Atranorin
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8. Linoleic Acid
9. Asiaticoside
10. Asiaticoid
11. 1β,2α-dihydro aleuritolic acid 2,3-*bis*-hydroxy benzoate.
12. 2α-hydroxy aleuritolic acid 3-*p*-hydroxy benzoate
13. 2α-hydroxy aleuritolic acid 2,3-bis-p-hydroxy benzoate.
14. Aleuritolic acid -3-*p*-hydroxy benzoate
15. Aleuritolic acid
16. Aleuritolic acid-3-acetate
17. Camaric acid
18. Lantanolic
19. Ursolic Acid
20. 2-α-hydroxyUrsolic acid
21 3β-o-coumaryl
22. 15α-hydroxy,β-amyrin
23. 3β-taraxerol
24. 3β-taroxerolfoemate
25. 3β-taraxerol acetate
26. 3β-o-(z)-coumaroyl-teroxerol
27. 3-β-o-(z)-coumaroyl-teroxerol
28. Oleanolic acid
29. Nigranoic Acid (3,4-secocycloarta-4(28),24-(Z)-diene-3,26-dioic acid)
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31. Olean-12-en-3,15diol
32. Olean-12-en-3,15,24 triol

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34. Camaldulensic acid
35. Camaldulenic acid
36. 3-o-coumaryl,15a-hydroxy-b-amyrin
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38. 12α-hydroxy-3,15-friedelindione
39. Stigmasterol
40. Beta amyrin
41. Friedelan-3β-ol
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43. 3β-hydroxy-cycloart-25-en-24-one
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46. Cycloart-23-ene-3β,25-diol
47. Cycloartenol
48. 24-methylenecycloartanol
49. Cycloart Glutinol
50. Lupanone
51. 3α-E-frruloylteraxerol
52. 3α-z- frruloylteraxerol
53. 3β-E-frruloylteraxerol
54. 3β-z- frruloylteraxerol
55. 3α-E-coumaroyltaraxerol
56. 3β-Z-coumaroyltaraxerol
57. 11,21-dioxo-2beta,3beta,15alpha-trihydroxyurs-12-ene-2-O-beta-D-glucupyranoside
58. 11,21-dioxo-3beta,3beta,15alpha,24-trihydroxyurs-12-ene-24-O-beta-D-glucupyranoside
59. 11,21-dioxo-3beta,3beta,15alpha,24-trihydroxyolean-12-ene-24-O-beta-D-glucupyranoside
60. 3α-E-coumaroyl lupeol
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68. 2α-bromolupanone
69. lup-1(2)-en-3-one
70. lupane-[2,3-C]-1́,2́,5́-Oxadiozole
71. Betulinic Acid
72. Methyl betulinate
73. Methyl dihydrobetulinate
74. 2,2-dibromomethyldihydrobetulinate
75. 2α-bromomethyldihydrobetulinate
76. 28-carbomethoxylup-1-(2)en-3-one
77. Dioxim of methyldihydrobetulinate
78. 28-Carbomethoxy lupan[2,3-C]-1́,2́,5́-Oxadiazole
79. Cerin
80. Friedelin

INTRODUCTION

Throughout history, mankind has always been interested in naturally occurring compounds from pre-biotic, microbial, plant and animal sources. Various extracts of different parts of plants have been widely used in folk medicines and perfumes as well as in food flavor and preservatives and are more commonly utilized in chronic diseases like cancer, diabetes and asthma [1].

The ancient Egyptians have described several useful preparations such as opium and castor oil. They also used "rotten bread" for treating infections which resembles our use of antibiotics from moulds and fungi [2].

The Chinese are considered as leaders in using natural products for healing. The oldest compilation of Chinese herbs is 'Shen Nung Pen Ts'ao', which lists 385 materials, 5267 medicinal herbs were used in China in 1967. One of the most famous herbs among them is the ginseng root. *Panax ginseng* is used for health maintenance and for the treatment of various diseases. Another popular folk drug is the extract of the *Ginkgo* tree, *Ginkgo biloba* which can improve memory and mental alertness [3-7].

During the 17th century, the Jesuit brought with them from South America the bark of the China tree for the treatment of malaria. In 1820, Pelletia and Caventou isolated from the China tree the active compound, quinine. American-Indians used the powerful hallucinogen, mescaline for a long period. The Indian hemp plant, *Cannabis sativa*, is being used since 3000 BC, and still used as marijuana or hashish [8].

Interest in alternatives to modern medicine was never been higher than it is now, and a large part of that interest revolves around the use of medicinal plants. Human beings have been dependent on plants for their health care needs since the beginning of civilization. Of the 2,50,000 higher plant species on earth, more than 80,000 are medicinal in nature.

About 5000 species are extensively used in traditional systems of medicine and are studied in some detail[9]. India has one of the richest ethno botanical traditions in the world with more than 7000 species of plants found in different agro-ecosystems and are used by various indigenous systems of medicine and industries. The plant parts utilized in medicines can be root, wood, bark, whole plant, fruit/seed, flower or leaf. Different types of chemical compounds are extracted from various parts of the

plants. Some of the compounds have medicinal properties and some are economically important.

Pharmaceutical research of several countries looking towards plant based agrochemicals and botanical pesticides. Eastern Himalayas is rich in flora constituting of many important plants having medicinal value. The tribal medicinal practice in the above region provided the evidence of the utilization of medicinal plants by the local people as a folk lore.

Triterpenoids (members of terpenoid family) having a C30 skeleton also called isoprenoids is a large and diverse class of naturally occurring organic chemical. Like terpenes, triterpenoids may be derived from five carbon isoprene units [10]. There are several ways of assembling and/or modification of isoprene units for preparation of derived triterpenoids. Some of the triterpenoids (member of Terpenes) have established medicinal properties. Terpenes are a large and varied class of hydrocarbon, produced primarily by a wide variety of plants, particularly conifers, though some insects such as termites, swallowtail, butterflies, etc. emit terpenes.

Because of the considerable proven range of medicinal properties the triterpene skeleton is the prime attraction of pharmaceutical industries. In the present work three medicinal plants (*Xanthoxylum budrunga*, *Bischofia javanica* and *Quarcus suber*) have been selected for extraction of triterpenoids, preparation of the suitable derivatives of the isolated compounds and to study the antimicrobial activity associated with them.

Considering the importance of the antimicrobial properties of triterpenoids and its derivatives the following objectives were taken into consideration in the present study.

1. Isolation and Characterization of natural terpenoids from selected plants of proven antimicrobial activity.
2. Preparation of some derivatives of the isolated terpenoids and their characterization.
3. Bioassay of the natural and derived triterpenoids against some important pathogen of economically important plants.
4. *In vivo* assessment of selected potential triterpenoids for their antimicrobial activity.

In concord with the line of the objectives stated above a review of literature was performed. The review was a selective one rather than a comprehensive and have been presented in chapter-I. Following chapter-I, Chapter-II deals with the isolation, characterization and bioassay of triterpenoids extracted from *Xanthoxylum budrunga*. Chapter-III also deals with the isolation, characterization and bioassay of triterpenoids extracted from *Bischofia javanica*. Chapter IV deals with isolation, characterization and preparation of two antimicrobial derivatives from the plant *Quarcus suber*, an herb commonly called Cork Oak, belongs to the family Fagaceae (Beech family).

* The scientific name of *Xanthoxylum budrunga* is changed to *Zanthoxylum budrunga*.

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■ CHAPTER - I

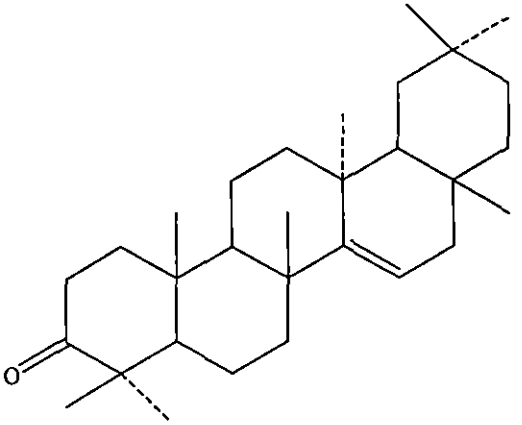
REVIEW OF LITERATURE

At the onset of the present study, it was considered worth while to review the work of the previous workers regarding triterpenoids in a selective manner. The observations of the previous workers have been presented briefly in the following paragraphs. For convenience, the observations have been divided into two subgroups which are as follows:

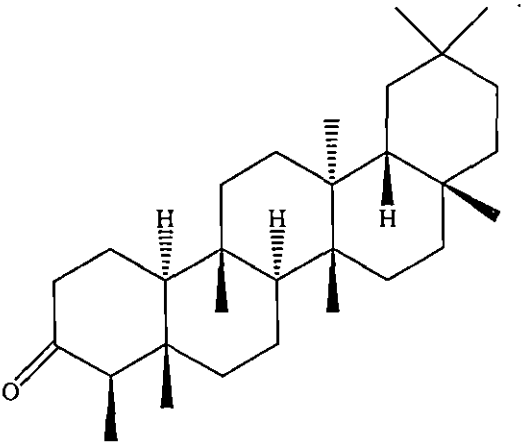
1. **Phytochemical investigations on medicinal plants containing triterpenoids as major constituents.**
2. **Potential antimicrobial activities of triterpenoids and their derivatives.**

1. Phytochemical investigation on medicinal plants containing Triterpenoids as major constituents

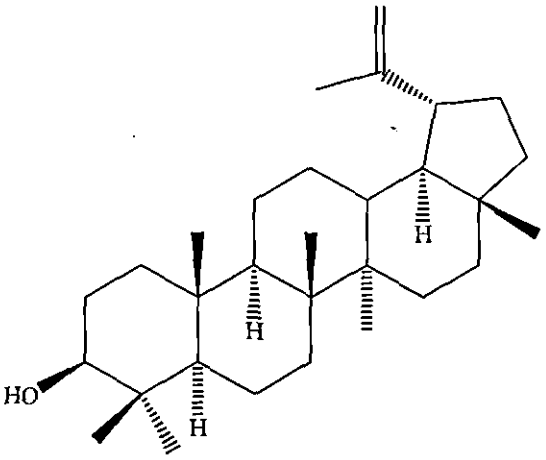
Gunaskera *et al.*[1] isolated new lupane derivative 3β–hydroxy-28-*p*-coumaroyloxy-lup-20(29)-27-oic acid from *Cassipa densifolia* and whose structure was deduced by chemical correlation with betulin, Simiarenol, taraxerone, friedelin, lupeol, betulinic acid, betulin, and β-sitosterol-g-D-glucoside.



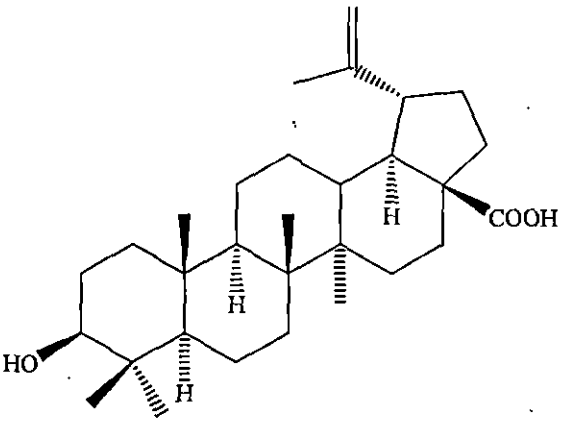
Taraxerone (1)



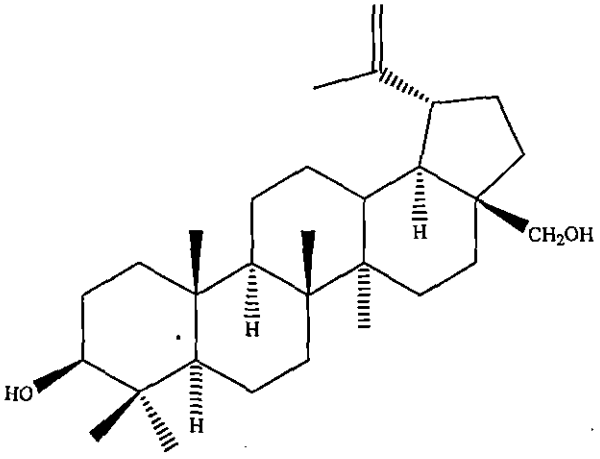
Friedelin (2)



Lupeol (3)

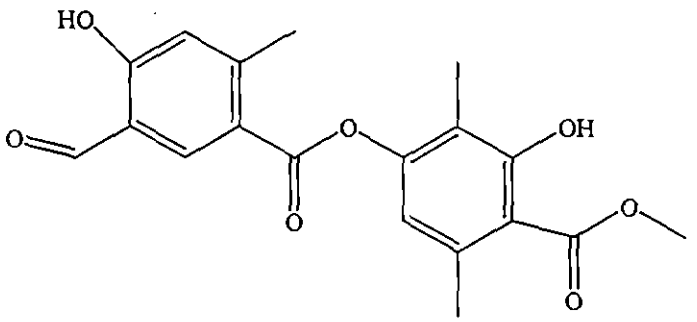


Betulinic acid (4)

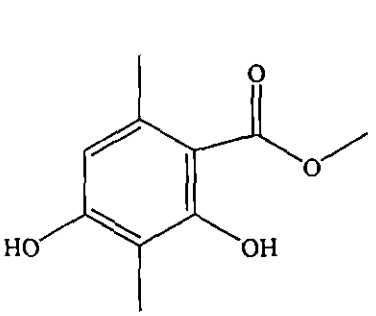


Betulin (5)

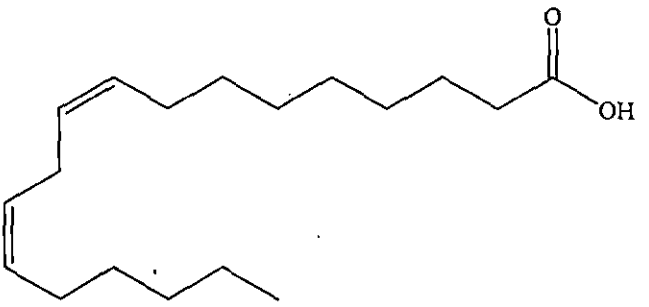
Mutai *et al.*[23] isolated three new pentacyclic triterpenoids: (20R)-3-oxolupan-30-al, (20*S*)-3-oxolupan-30-al and (20*R*)-28-hydroxylupen-30-al-3-one, along with (20*S*)-3β-hydroxylupan-30-al and the known metabolites 30-hydroxylup-20-(29)-en-3-one, 30-hydroxylup-20-(29)-en-3β-ol, atranorin, methyl2,4-dihydroxy-3,6-dimethylbenzoate, sitosterol-3β-O-glucoside and linoleic acid from *Acacia mellifera* . The structures of the new metabolites were elucidated by extensive spectroscopic analyses and their relative stereochemistry was determined by NOESY experiments. They observed that the new metabolite 3 exhibited significant cytotoxic activity against the NSCLC-N6 cell line, derived from a human non-small-cell bronchopulmonary carcinoma.



Atranorin (6)

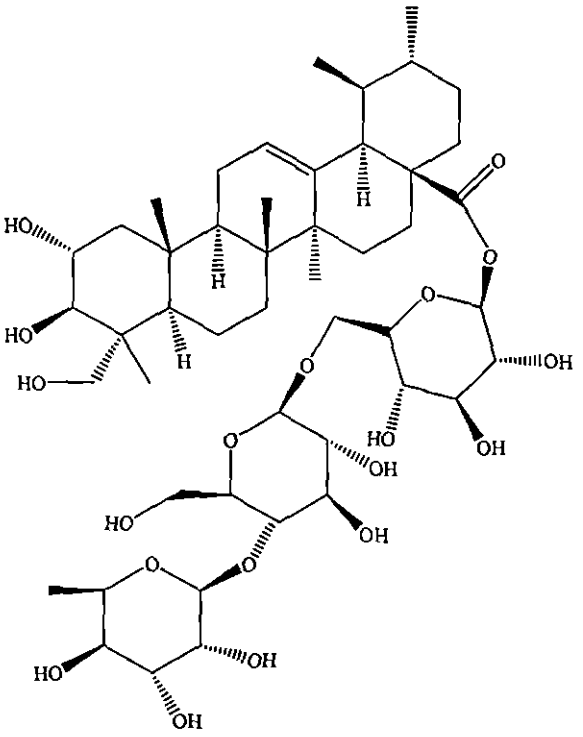


Methy-l2,4-dihydroxy-3,6-dimethylbenzoate (7)

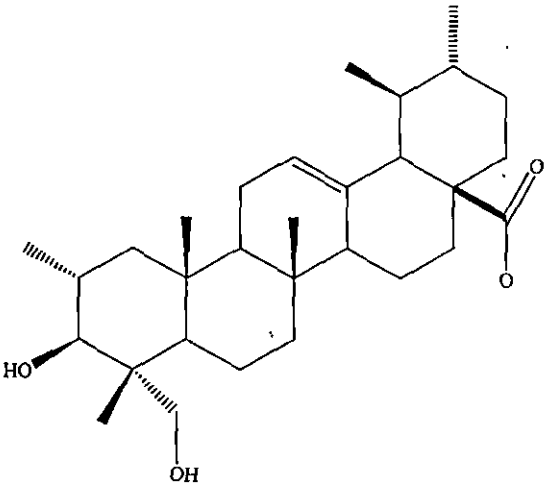


Linoleicacid (8)

James and Dubery[3] accumulated large quantities of pentacyclic triterpenoid saponins collectively known as centelloids from *Centella asiatica*. These terpenoids include asiaticoside, centelloside, madecassoside, brahmoside, brahminoside, thankuniside, sceffoleoside, centellose, asiatic-, brahmic-, centellic- and madecassic acids. They studied biological activity of these compounds, the Centella triterpenoids can be regarded as phytoanticipins due to their antimicrobial activities and protective role against attempted pathogen infections. They reported that these plant-derived pharmacologically active compounds have complex structures; the production of secondary metabolites by cultured cell provided a particular important benefit of manipulation and improved the production of the desired compounds.



Asiaticoside (9)



Asiatic acid(10)

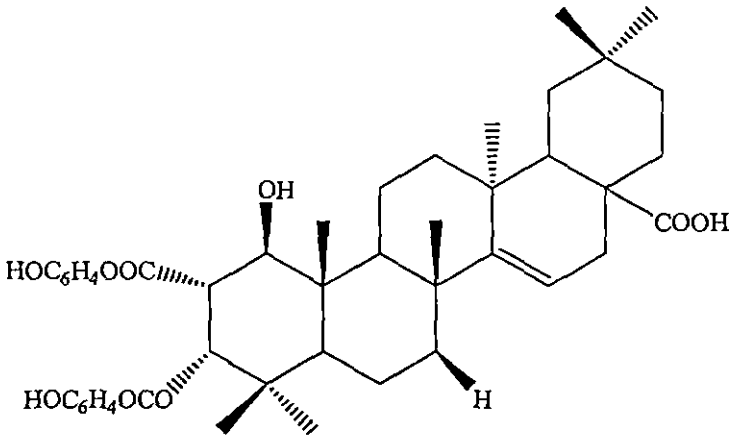
Antonia *et al.*[4] extracted lupane triterpenoid lupeol, the ursane triterpenoid α–amyrin and esters of these compounds from the bark of roots of *Alstonia boonei* and observed that those compounds had anti-inflammatory properties. They found that α–amyrin was a competitive inhibitor of bovine trypsin and chymotrypsin; lupeol linoleate, lupeol palmitate and α–amyrin linoleate were non-competitive inhibitor of chymotrypsin. They also found that lupeol, α–amyrin, palmitic and linoleic acid esters of these compounds were very weak inhibitors of porcine pancreatic elastase and of *Lucilia cuprina* and *Helicoverpa punctigera* leucine aminopeptidases.

Li *et al.*[5] extracted a new lupane type triterpenoid, 3β, 11α–dihydroxy-30-norlupan-20-one and six known lupane triterpenoids from the whole plant of *Salvia roborowskii maxim* using petroleum ether as a solvent. They elucidated their structures by means of spectral methods including NMR and MS techniques.

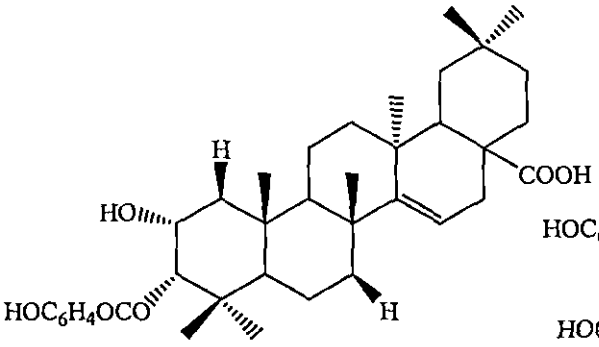
Tostikoya *et al.*[6] studied the biological activity of natural and semi synthetic lupane triterpenoid and discussed in two-part review. The first part was devoted to the pharmacological properties of natural lupane triterpenoids. They reported that betulinic acid proved to be the most effective antitumor agent among more than fifty natural lupines.

Kanokmedhakul *et al.*[7] isolated a new 1,3-dihydroxy-2-methyl-5,6-dimethoxyanthraquinone [a], six known anthraquinones [b], nordamnacanthal [c], damnacanthal ,rubiadin [d], rubiadin-1-methylether [e], lucidin-ω-methylether [f] and 1-hydroxy-2-hydroxymethyl-3-methoxyanthraquinone [g], a β-sitosterol, together with two known triterpenoids, β-acetylolean-12-en-28-olicacid [h] and 3β-O-acetyl-11α,12α-epoxyolean-28,13-olide [i] from the roots and stems of *Prismatomeris fragrans*. Their structures were established on the basis of spectral data. They studied the antiplasmodial, antituberculosis, antifungal and anticancer cell lines tests of the isolated compounds and the bioactivity assays showed that only **i** exhibited moderate antimalarial activity, **b** and **c** exhibited antifungal activity while **b, c, d, g** and **i** showed antituberculosis activity. In addition, compounds **b, c** and **g** exhibited cytotoxicity to BC cell line while **a,** (the methyl ether derivative of **1**), **b, c, d, e,** and **i** exhibited cytotoxicity to NCI-H187 cell line.

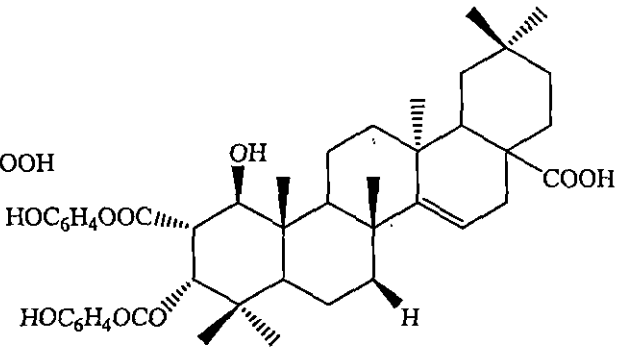
Chaudhuri *et al.*[8] isolated pentacyclic triterpenoids based on the taraxer-14-ene skeleton with a C-28 attached carboxylic acid group from the roots of *Maprounea africana*. They identified these compounds as 1β, 2α-dihydroxyaleuritolic acid 2,3-bis-hydroxybenzoate,2α-hydroxyaleuritolic acid 3-*p*-hydroxybenzoate, 2α-hydroxyaleuritolic acid 2,3-bis-*p*-hydroxybenzoate, aleuritolic acid 3-*p*-hydroxybenzoate, aleuritolic acid, and aleuritolic acid 3-acetate. They reported that compounds 1β, 2α-dihydroxyaleuritolic acid 2,3-bis-hydroxybenzoate, were new triterpene esters.



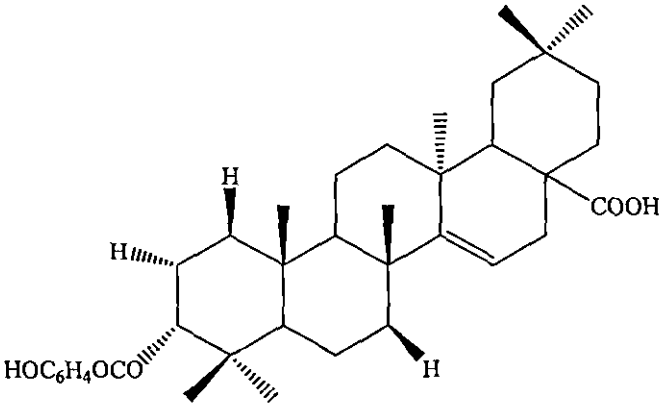
1β, 2α-dihydroxyaleuritolic acid 2,3-bis-hydroxybenzoate (11)



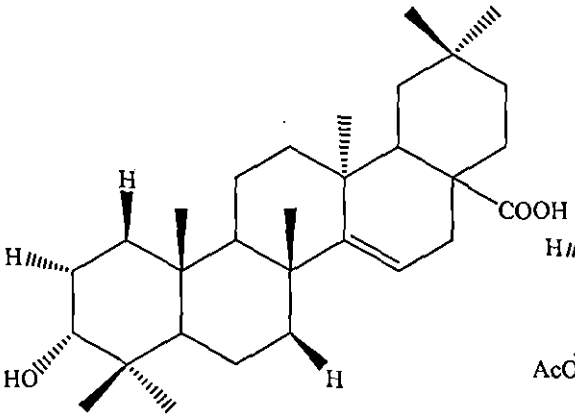
2α-hydroxyaleuritolic acid 3-p-hydroxybenzoate (12)



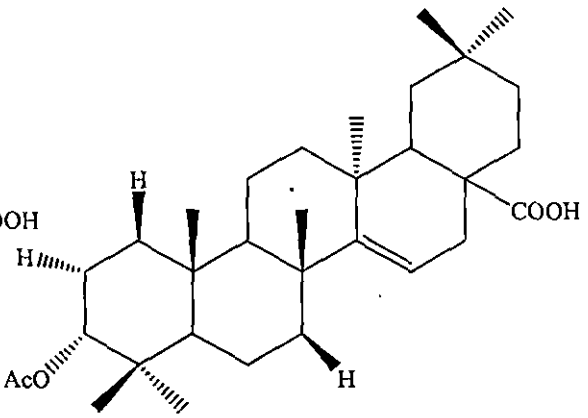
2α-hydroxyaleuritolic acid 2,3-bis-p-hydroxy benzoate(13)



Aleuritolic acid-3-p-hydroxybenzoate (14)

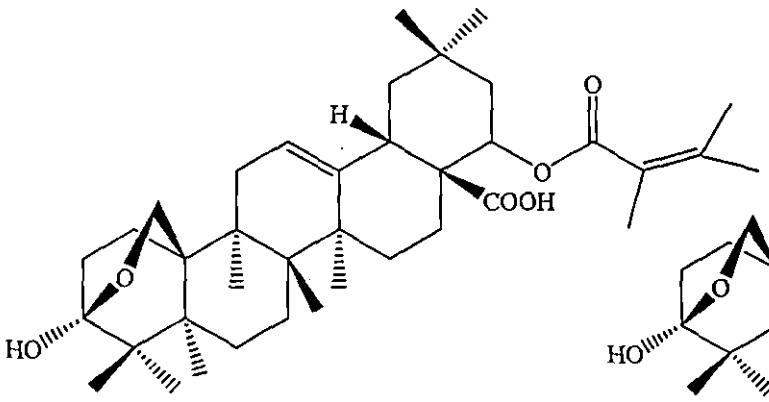


Aleuritolic acid (15)

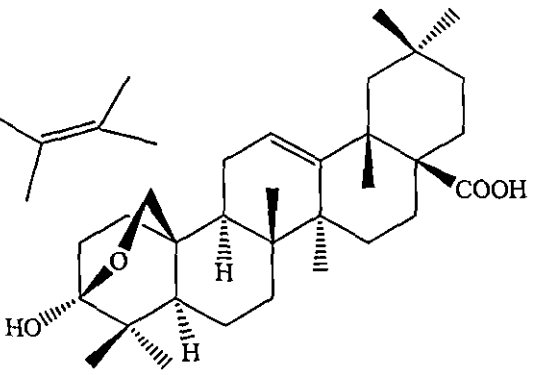


Aleuritolic acid 3-acetate (16)

Begum *et al.*[9] isolated three new pentacyclic triterpenoids, camaryoloic acid, methylcamaralate and camangeloyl acid with six known compounds, β-sitosterol 3-O-β-D-glucopyranoside, octadecanoic acid, docosanic acid, palmitic acid, camaric acid and lantanolic acid from the aerial parts of *Lantana camara*. They elucidated the structures of the new compounds by spectroscopic and chemical methods.



Camaric acid (17)



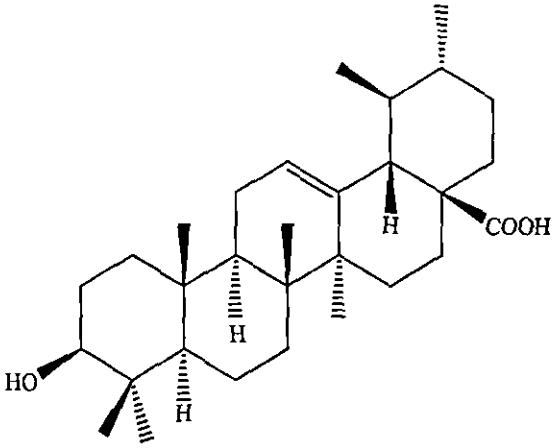
lantanolic acod (18)

Rahaman *et al.*[10] reported that two new triterpenoids, 18α,19 β-20(30)taraxasten-3β,21α-diol (cichoridol) and 17-epi-methyl-6-hydroxyangolensate (intybusoloid) obtained from the methanolic extract of seeds of *Cichorium intybus* (Asteracae) along with eleven known compounds, lupeol, friedelin, betunaldehyde, syrginic acid, vanillic acid, 6,7-dihydroxycoumarin and methyl-alpha-D-galactopyranoside Compound.

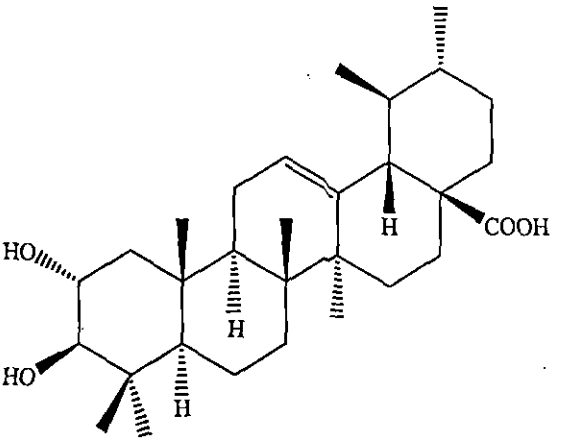
Choudhury *et al.*[11] reported the biotransformation of a pentacyclic triterpene, oleanolic acid, with *Fusarium lini* afforded two oxidative metabolites, 2α,3β–dihydroxy-olean-12-en-28-oic acid, and 2α, 3β, 11β–trihydroxyolean-12-en-28-oic acid. They also found that metabolite 3 was a new compound. The structures were characterized on the basis of spectroscopic studies. These metabolites exhibited a potent inhibition of α–glucosidase enzyme and thus were effective in diabetes by delaying the glucose absorption.

Begum *et al.*[12] isolated two triterpenoids, 20β-acetoxy-2a,3β-dihydroxyrus-12-en-28-oic acid (guavanoic acid, 3) and 2α,3β-dihydroxy-24-*p*-z-coumaroyloxyrus-12-en-28-oic acid (guavacoumaric acid, along with six known compounds such as 2α-hydroxyursolic acid, jacoumaric acid, isoneriucoumaric acid, asiatic acid, ilelatifol D and β-sitosterol-3-O-β-D-glucopyranoside from the leaves of *Psidium guajava*. They determined the structures of the isolated compounds through spectroscopic methods.

Shai *et al.*[13] isolated four compounds (lupeol, betulinic acid, ursolic acid and 2 alpha-hydroxyursolic acid) from the leaves of *Curtisia dentata*. They studied the antibacterial and antifungal activity (using broth microdilution assay and bioautography method) and found that betulinic acid, ursolic acid and 2α-hydroxyursolic acid appreciably inhibited fungal growth with MIC values ranged between 8 to 63 μg/mL.



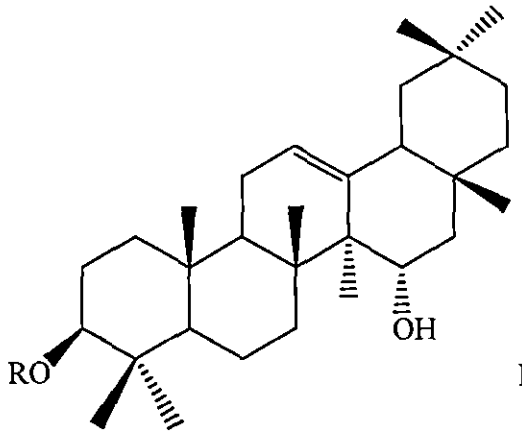
Ursolic acid (19)

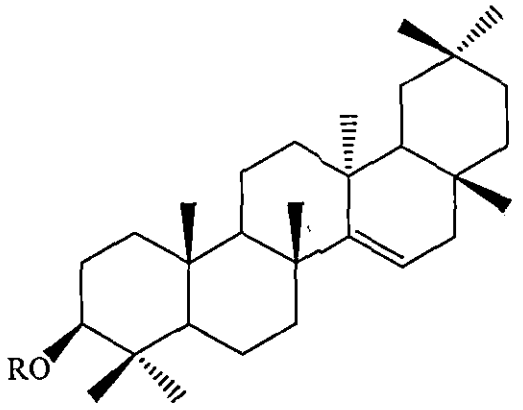


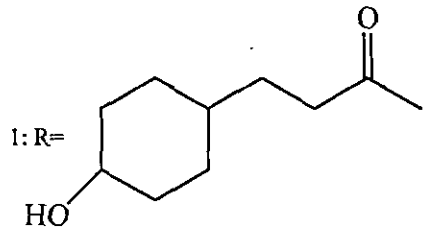
2-α-hydroxyursolic acid (20)

Gu *et al.*[14] derived three pentacyclic triterpenoids from *Valeriana laxiflora* DC ,*Lavandula dentate* L.,*Tanacetum parthenium* (L.) Sch. Bip.as oleanolic acid, betulinic acid and ursolic acid and found that the isolated triterpenoids were moderate anti-tubercular in a microplate alamar blue assay.

Li *et al.*[15] isolated seven pentacyclic triterpenoids including 3β–O–coumaryl-β–amyrin [fig.21], 15α–hydroxy, β–amyrin [fig.22], 3β–taraxerol [fig.23], 3β–taraxerol formate [fig.24], 3β–taraxerolacetate [fig.25], 3β–O–(E)–coumaryl-taraxerol [fig.26] and 3-β-*o*-(Z)-coumaroyl-taraxerol [fig.27] from the stems and twigs of the mangrove plant *Rhizophora stylosa* (Rhizophoraceae). The structures of the isolated compounds were determined by extensive analysis of their spectroscopic data. Among the metabolites, compound **1** was a new olenane type terpenoid coumaroyl ester, while compound **4** was a new natural product.







21: R = 3β–O–coumaryl

22. R=H=15α–hydroxy, β–amyrin

23. R=H=3β–taraxerol

24. R=Formyl=3β–taraxerol formate

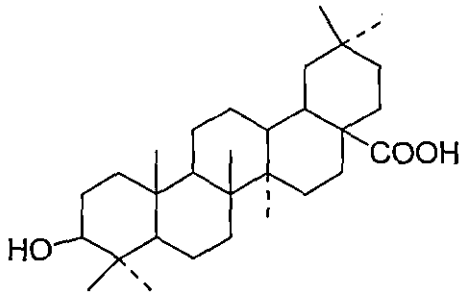
25. R=Acetyl=3β–taraxerol acetate

26. R=E-coumaryl=3β–*o*–(E)–coumaryl-taraxerol

27. R=Z-coumaryl=3–β-*o*- (Z)-coumaroyl-taraxerol

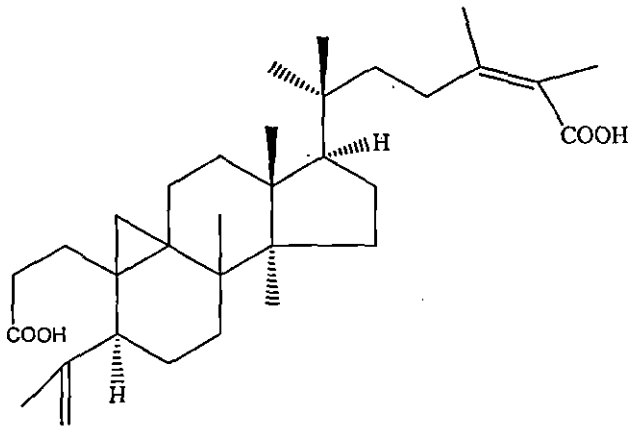
Pichai *et al.*[163] extracted the powdered material of "Vidattali" equated to *Dichrostachys cinerea* and separated n-octacosanol, β–sitosterol, friedelin, epifridelinol, α-amyrin and β–sitosterol-3-β-D-glucopyranoside from the aerial part. They studied antibacterial and antifungal activities of n-hexane and chloroform extracts on three bacteria (*Escherichia coli*, *Pseudomonas aeruginasa* and *Staphylococcus aureus*) and two fungi (*Aspergillus flavus* and *Mucor* sp.) at 1.25, 2.5, 5.0 and 10 mg/mL concentrations. Study was made in nutrient agar and SDA mediums by steak method. They observed that the chloroform extract showed moderate antibacterial efficacy towards *E.coli* and

Staphylococcus at higher concentrations (5-10 mg/mL). Antifungal activities of the extracts against *Aspergillus* and *Mucor* were also observed at higher concentrations.

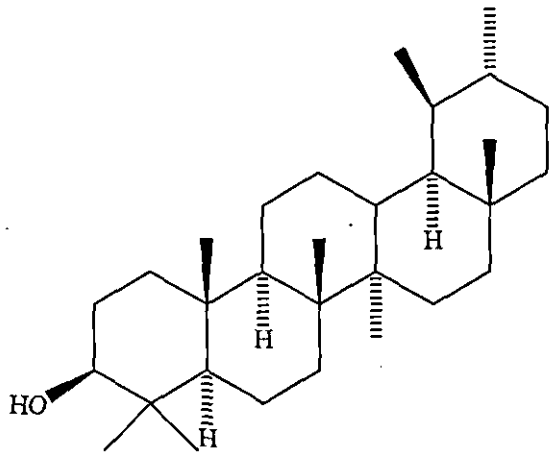


Oleanolic acid (28)

Sun *et al.*[17] isolated a ring-secocycloartene triterpenoid, nigranoic acid (3,4-secocycloarta-4(28),24-(Z)-diene-3,26-dioic acid from the stem of *Schisandra sphaerandra*, a Chinese traditional medicinal plant. The structure elucidation and unambiguous NMR spectral assignment were achieved by the combination of 1D and 2D-NMR techniques with the aid of computer modeling. They found that nigranoic acid showed activity in several anti-HIV reverse transcriptase and polymerase assays.



Nigranoic acid (3,4-secocycloarta-4(28),24-(Z)-diene-3,26-dioic acid (29)



α-amyrin (30)

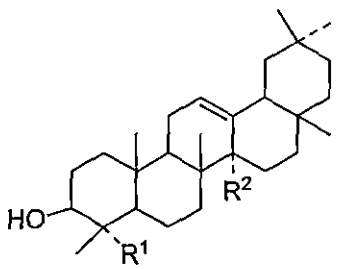
Chi:ozem *et al.*[18] isolated two new friedelane-type triterpenes named 12-alpha-hydroxyfridelane-3,15-dione and 3-beta-hydroxyfriedelan-25-al, together with six known compounds from the stems of *Drypetes paxii* Hutch (Euphorbiaceae) and established their structures. They also tested the antimicrobial activity of the five friedelane-type triterpenes, one olean-12-ene triterpene saponin against some Gram-positive and Gram-negative bacteria and they appeared to be modestly active.

Angeh *et al.*[19] isolated four known triterpenoids, 11-alpha,3-beta-dihydroxy-12-oleanen-29-oic acid, 1-hydroxy-12-olean-30-oic acid, 3,30-dihydroxyl-12-oleanen-22-one and 1,3,24-trihydroxyl-12-olean-29-oic acid along with a new pentacyclic triterpenoid (1-alpha, 23-dihydroxy-12-oleanen-29-oicacid-3beta-O-2, 4-di-acetyl-L rhamnopyranoside) through a bioassay-guided procedure from the leaves of *Combretum imberbe*. The structures of the compounds were elucidated on the basis of 1D and 2D NMR experiments, as well as mass spectrometric data. They observed that all the isolated compounds have moderate (62 μg/mL) to strong (16 μg/mL) antibacterial activity (MIC values) against *Staphylococcus aureus* and *Escherichia coli*, with 1 and 5 being most active. The results of the study gave credence to the ethnomedicinal use of *Combretum imberbe*.

Mathabe *et al.*[20] extracted four known compounds, two triterpenoids, compound 1 [d-friedoolean-14-en-oic acid (3-acetyl aleuritolic acid)] and compound 2 (lupeol), and two diterpenes, compound 3 [2,6alpha-dihydroxy-norbeyer-1,4,15-trien-3-

one (diosphenol 2)] and compound 4 (3beta-hydroxy-beyer-15-ene-2-one) from the bark of *Spirostachys africana* using ethanol as a solvent . They tested the antibacterial activity of the isolated compounds using micro-dilution method Compound 1, exhibited minimum inhibitory concentration (MIC) of 50μg/mL against *Staphylococcus aureus, Salmonella typhi, Vibrio cholera, Escherichia coli* and *Shigella dysentery.*

Wada *et al.*[21] isolated lupane and oleanane type triterpenoids from the bark of *Phyllanthus flexusus* and screened inhibitory activity on human Topos (topoisomerases) I and II. They found that olean-12-en-3β, 15α-diol, olean-12-en-3β, 15α, 24-triol, lupeol, and betulin were selective catalytic inhibitors of human Topo II activity with IC_{50} values in the range of 10-39 μM/mL.

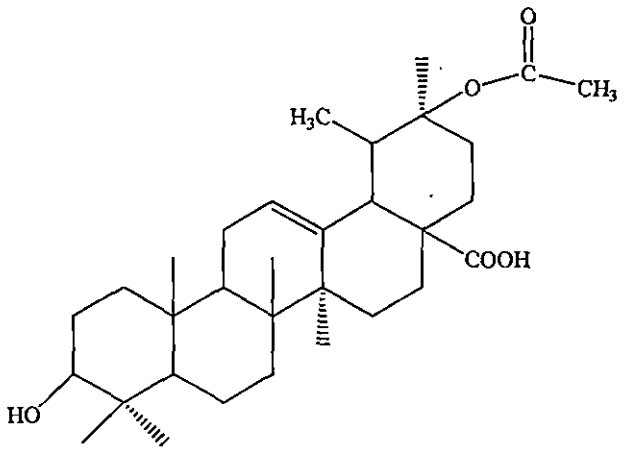


	R^1	R^2	
31	Me	OH	= olean-12-en-3,15-diol
32	CH_2OH	OH	= olean-12-en-3, 15, 24-triol

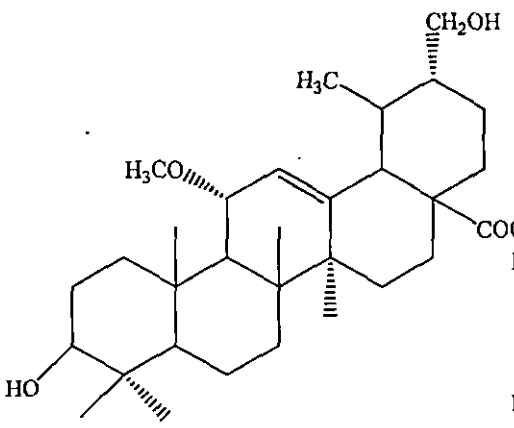
Siddiqui *et al.*[22] investigated the constituents of the fresh, uncrushed leaves of *Eucalyptus camaldulensis* var. *obtusa* and isolated a triterpenoid (amirinic acid) with four known triterpenoids ursolic acid lactone, betulinic acid, oleanolic acid and ursolic acid. They transformed amirinic acid into amirolide in deuterated chloroform at room temperature. The new products were characterized by exhaustive spectroscopic studies.

Siddiqui *et al.*[23] studied the fresh leaves of *Carissa carandas* collected from the Karachi Region in Pakistan and isolated four pentacyclic triterpenoids including one new constituent carissin and two hither to unreported compounds. They elucidated the structure of the new compound as 3beta-hydroxy-27-E-feruloyloxyurs-12-en-28-oic acid.

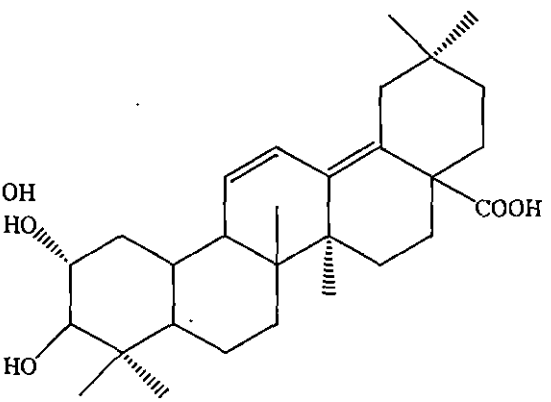
Begum and Siddiqui[24] investigated the constituents of fresh, uncrushed leaves of *E. camaldulensis* var. *obtuse* and isolated a known and 3 new triterpenoids. They characterized the new compounds by chemical and spectroscopic studies as camaldulic acid (20 beta-acetoxy-3 beta-hydroxyrus-12-en-28-oic acid), camaldulensic acid (3-beta, 30-dihydroxy-11 alpha-methoxyrus-12 en-28-oic acid) and camaldulenic acid (2-alpha, 3-beta-dihydroxyolean-11,13(18)-dien-28-oic acid)



Camaldulic acid (33)



Camaldulensic acid (34)



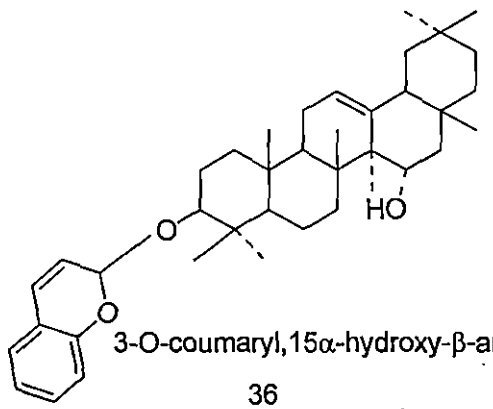
Camaldulenic acid (35)

Setzer *et al.*[25] extracted the crude from the bark of *Syncarpia glomulifera* using chloroform as a solvent and reported antibacterial and cytotoxic activity. They isolated

oleanolic acid-3-acetate, ursolic acid-3-acetate and betulinic acid from the bark. They observed that the relatively large abundance (10 % of the crude extract) and high degree of activity of betulinic acid were responsible for the bioactivity of the crude bark extract.

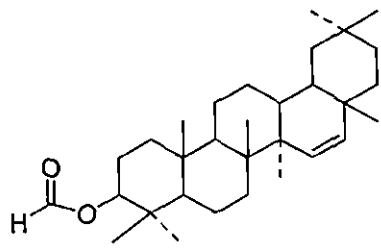
Lutskii *et al.*[26] isolated triterpenoids from the plants of the *Thalictrum sp* and the structural, chemical and spectral properties were systematized for the first time. They discussed the features of the ^{13}C NMR spectra of cycloartane triterpenoids.

Li *et al.*[27] isolated seven pentacyclic triterpenoids including 3β-*O*-(*E*)-coumaroyl-15α-hydroxy-β-amyrin [fig.36], 15α–hydroxy-β-amyrin, 3β-taraxerol, 3β-taraxerol formate [fig.37], 3β-taraxerol acetate [fig.38], 3β-*O*-(E)-coumaroyl-taraxerol [fig.39], and 3β-*O*-(*Z*)-coumaroyl-taraxerol from the stems and twigs of the mangrove plant *Rhizophora stylosa*. The structures of the isolated compounds were determined by extensive analysis of their spectroscopic data. They reported that among these metabolites, compound fig.36 was a new oleanane-type triterpenoid coumaroyl ester, while compound fig.37 was a new natural product obtained here as an isolated substance for the first time.

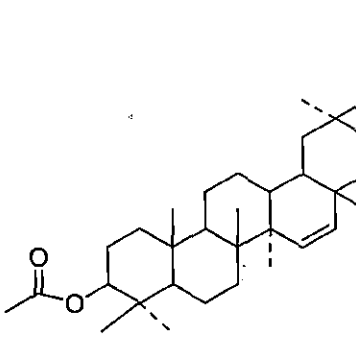


3-O-coumaryl,15α-hydroxy-β-amyrin

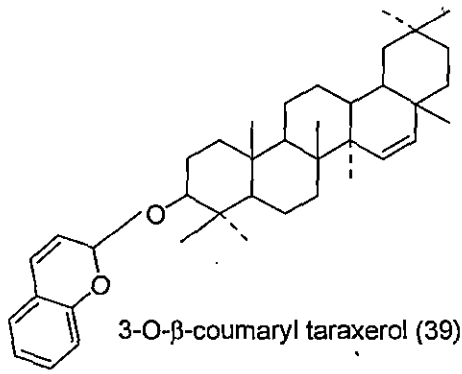
36



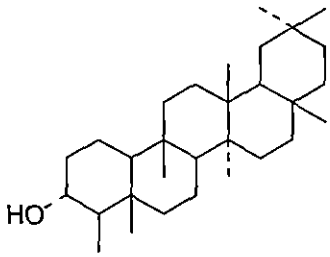
3β-taraxerol formate (37)



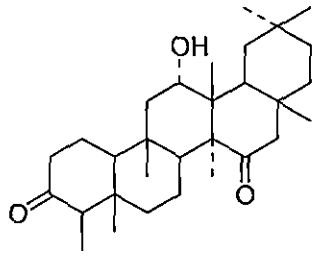
3β-taraxerol acetate (38)



3-O-β-coumaryl taraxerol (39)



3-epifridelinol (40)



12α–hydroxy-3,15-friedelin dione (41)

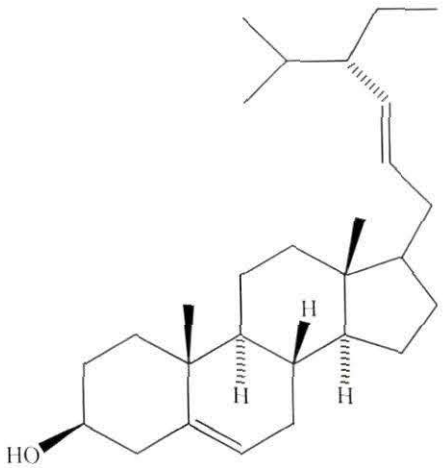
Xu *et al.*[28], isolated Geumonoid, a new triterpene from *Geum japonicum* and its structure was elucidated on the basis of 1D, 2D NMR and MS spectroscopic analysis. They observed that Geumonoid showed inhibitory activity against HIV-1 protease.

He *et al.*[29], isolated the chemical constituents of the roots of *Aconitum taipaicum* (Ranunculaceae) and purified using silica gel column chromatography. They found new norditerpenoid alkaloids, isodelelatine along with five known alkaloids. The structure of the new compound was elucidated on the basis of spectral data.

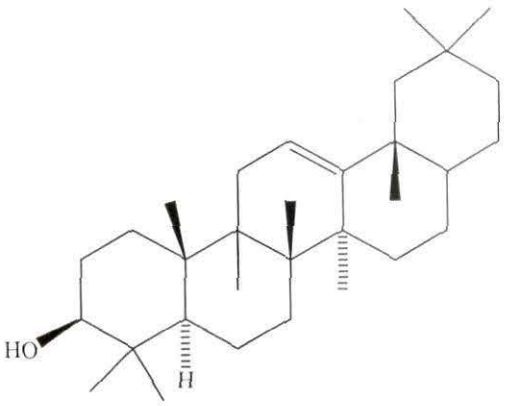
Srikrishna *et al.*[30] carried out antibacterial activity using cup plate method by petroleum ether, chloroform, methanol and water extract of the bark of *Aporosa lindleyana* (Euphorbiaceae). They observed that the compounds showed moderate to very good activity against *Bacillus subtilis, Escherichia coli* and the data were compared with the standard drug tetracycline. They studied the antifungal activity against *Penicillium chrysogenous, Candida albicans, Aspergilus niger* and *Trichoderma viridi* and compared with the standard drug fluconazole. The petroleum ether extract showed considerable

activity towards all the four fungal organisms. Analgesic activity has been carried out on Swiss albino male mice by abdominal constriction method. All the extracts showed

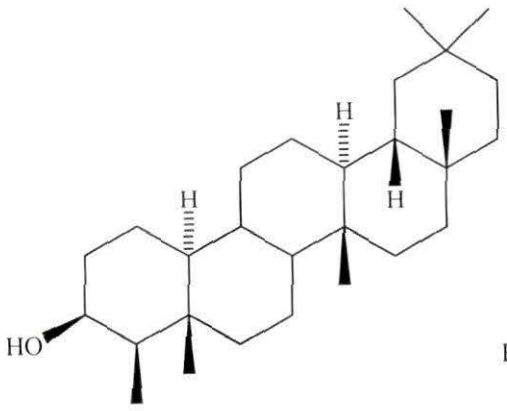
Singh *et al.*[31] extracted a mixture of steroids and triterpenoids: β–sitosterol, stigmasterol, β–amyrin, friedelan-3β–ol (epifridelenol), cycloartenone, β–amyrin acetate, friedelin and epi-friedenyl acetate from *Heliotropium marifolium* using hexane as a solvent. They tested the isolated triterpenoids against selected pathogenic bacteria and fungi, e.g. *Escherichia coli, Staphylococcus aureus, Aspergillus niger and Penicillium chrysogenum.* They also discussed the quantification and assessment of their growth inhibitory potency and found that cycloartenone was the major triterpenoids in both *in vivo* and *in vitro* cell culture.



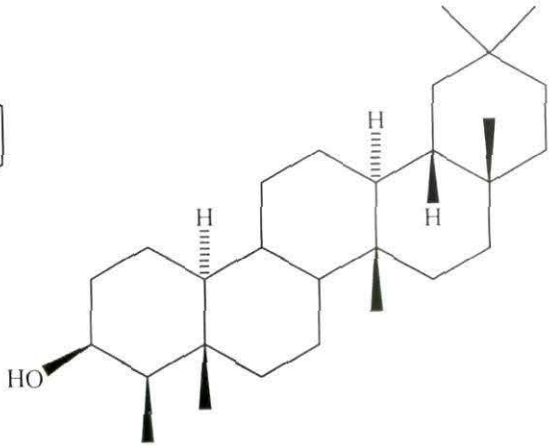
Stigmasterol (42)



Betaamyrin (43)



Friedelan-3β-ol (44)



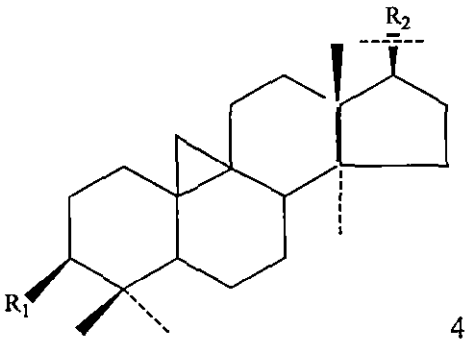
3β-hydroxy friedilane (45)

Sukul and Chaudhuri[32], extracted the leaves of *Lantana camara* using different solvents. They observed that four fractions of petroleum ether extract showing significant antibacterial activity against some human pathogens under *in vitro* conditions. The MIC of the methanol fraction, containing triterpenoids, active against these pathogens was found to be comparable with those of some therapeutically used antibiotics.

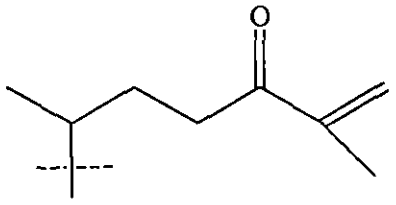
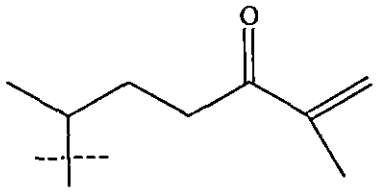
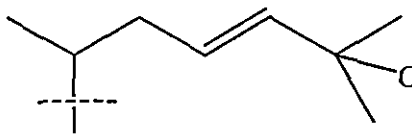
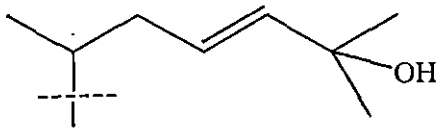
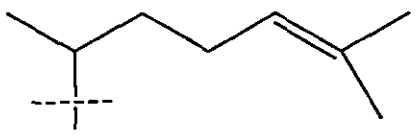
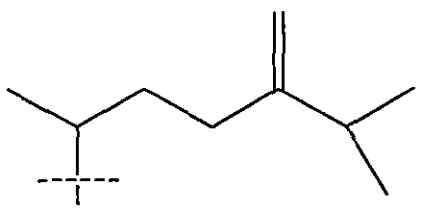
Panizzi *et al.*[33] isolated some constituents from the flowering aerial parts of *Geum rivale* and studied their antimicrobial activity on bacteria and fungi. The activity was more in the triterpene fractions for Gram-positive and Gram-negative bacteria; activity was also toward flavonoid fractions.

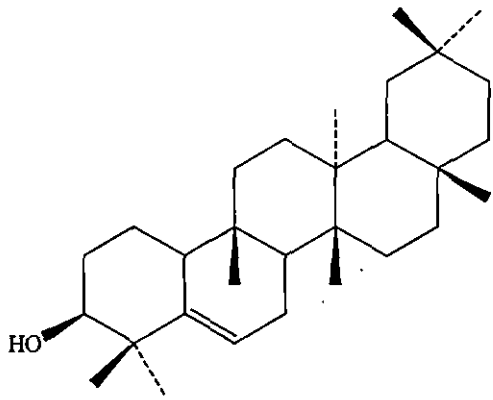
Takeoka *et al.*[34] isolated three triterpenoids betulinic acid, oleanolic acid and ursolic acid as their methyl esters from diethyl ether extracts of almond hulls using flash chromatography and preparative high performance liquid chromatography. They were characterized triterpenoids using chromatographic and spectroscopic methods and these studies demonstrated that almonds hulls were a rich source of triterpenoids. They reported anti-inflammatory, anti-HIV and anti-cancer activities of these triterpenoids.

Madureira *et al.*[35] carried out phytochemical re-investigation of the whole plant of *Euphorbia segetalis* and isolated five tetracyclic triterpenes: 3β-hydroxy-cycloart-25-en-24-one, cycloart-25-ene-3β,24-diol, cycloart-23-ene-3β,25-diol, lanosta-7,9(11),24-trien-3β-ol and lanosta-7,9(11),24(31)-trien-3β-ol, 3β-acetoxy-cycloart-25-en-24-one and glutinol, lupenone, friedelin dammaranodienol, cycloartenol acetate, 24-methylenecycloartanol acetate and β-sitosterol. They were studied for their antiviral activities against Herpes simplex virus (HSV) and African swine fever virus (ASFV) and observed that lupenone exhibited strong viral plaque inhibitory effect against HSV-1 and HSV-2. The *in vitro* antifungal and antibacterial activities of cycloart-23-ene-3β,25-diol, was also investigated.



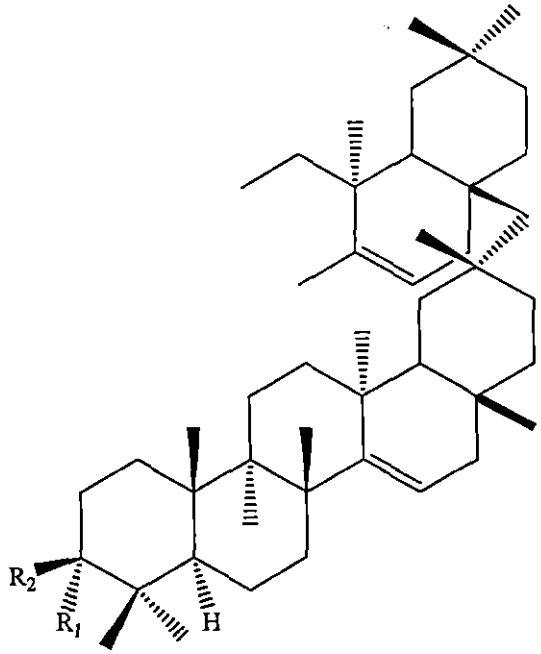
46-51

R_1	R_2	
OH		= 3 βl-hydroxy-cycloart-25-en-24-*one*
OAc		= 3β-acetoxy-cycloart-25-en-24-one
OH		= cycloart-25-ene-3β,24-diol
OAc		= cycloart-23-ene-3β,25-diol
OH		= cycloartenol
OH		= 24-methylenecycloartanol



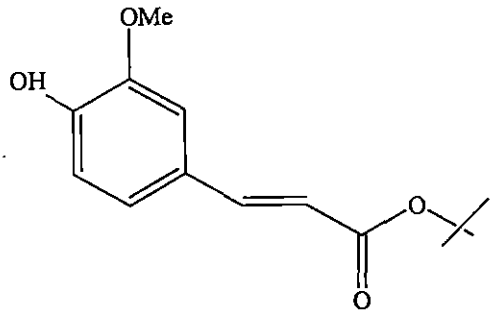
Glutinol (52)

Laphookhieo *et al.*[36] isolated six new pentacyclic triterpenoids esters (fig.53-58) together with 3α- and 3β-tarexerol from the fruits of *Bruguiera cylindrica*. The structures of the new compounds were characterized as 3α-E-feruloyltaraxerol (fig.53), 3α-Z-feruloyltaraxerol (fig.54), 3 β-E-feruloyltaraxerol (fig.55), 3 β–Z-feruloyltaraxerol (fig.56), 3α-E-coumaroyltararaxerol (fig.57), and 3α-Z-coumaroyltaraxerol (fig.58). They reported that compounds 2 and 6 exhibited weak cytotoxicity against the NCl-H187 cell line.



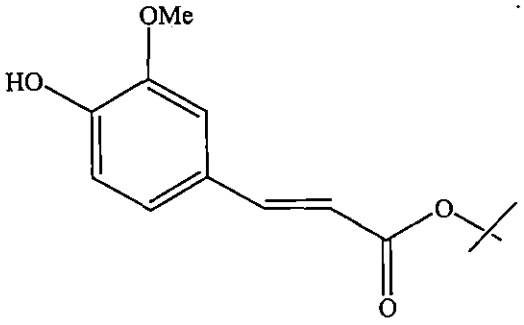
53

R_1 R_2

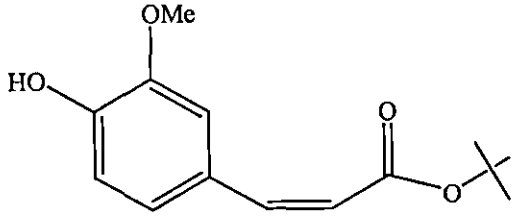


H = 3α–E-feruloyltaraxerol

54

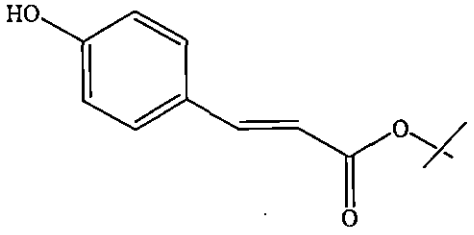


H = 3β–E-feruloyltaraxerol

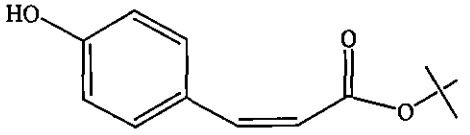


H =3β–Z-feruloyltaraxerol

55,56



H = 3α–E–coumaroyltaraxerol

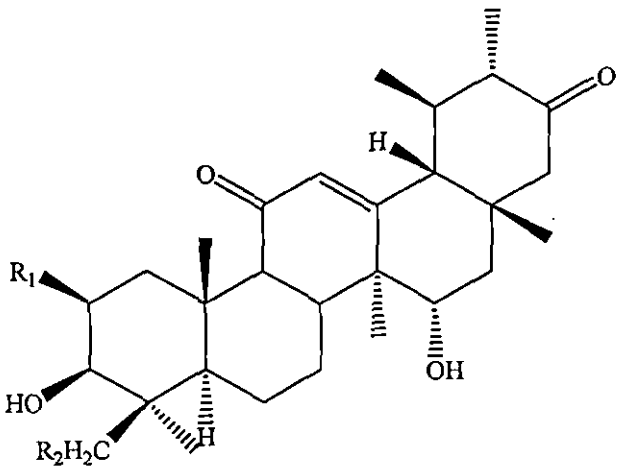


H =3β–Z–coumaroyltaraxerol

57,58

Araujo and Chaves [37] isolated eleven oleanane, ursane and lupane-type triterpenes daturadiol (3β,6β–dihydroxy-olean-12-ene), 3β-hydroxy-30-norlupan-20-one, lupenone, β-amyrenone, α–amyrenone, lupeol, β-amyrin, α–amyrin, betulin, erythrodiol and uvaol, in addition to squalene, sitosterol and α–tocopherol from the leaves of *Terminalia brasiliensis* Camb. They identified the structures of these compounds by ^{1}H and ^{13}C NMR spectral analysis.

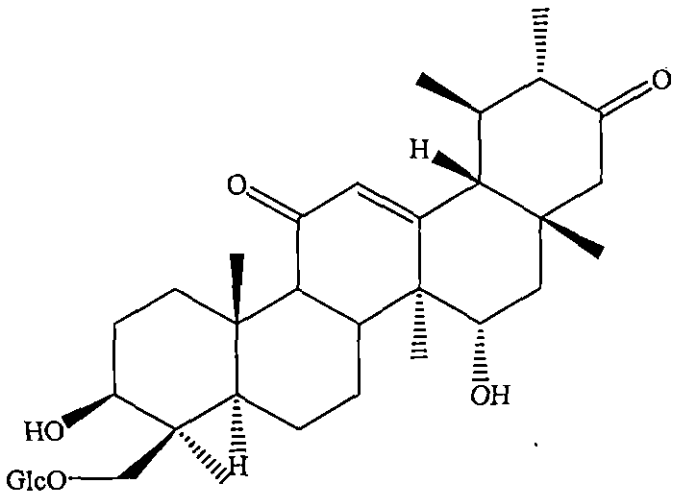
Ghosh *et al.*[38] extracted seeds of *Swietenia mahagoni* using methanol as a solvent and reported pharmacological activity including anti-inflammatory activity of the extract. They evaluated the anti-inflammatory activity using acute, sub-chronic, and chronic models of inflammation in rodents. The antipyretic and analgesic activities were evaluated in mice models. They studied the acute toxicity of the extract using different doses and the effect was compared with the standard drug, ibuprofen. The results revealed that the extract produces anti-inflammatory activity through dual inhibition. Zhou *et al.*[39] isolated three new triterpenoids: 11,21-dioxo-2beta,3beta, 15alpha-trihydroxyurs-12-ene-2-O-beta-D-glucopyranoside,11,21-dioxo-3-beta,15-alpha, 24-trihydroxyurs-12-ene-24-O-beta-D-glucopyranoside, and 11,21-dioxo 3-beta,15-alpha,24-trihydroxyolean-12-ene-24-O-beta-D-glucopyranoside, and two new flavonoids viz.apigenin-7-O-[2"-O-(5"'-O-feruloyl)-beta-D-apiofuranosyl]-beta-glucopyranoside and chrysoeriol-7-O-[2"-O-(5"'-O-feruloyl)-beta-D-apiofuranosyl]-beta-d-glucopyranoside from the whole plant of fresh *Apium graveolens* together with 10 known flavonoids. The structures of the new compounds were elucidated by analysis of spectroscopic data. They evaluated the inhibitory effects of the compounds isolated on nitric oxide production in lipopolysaccaride-activated macrophages.



1 R_1=OGlc, R_2=H ; 11,21-dioxo-2beta,3beta,15alpha-trihydroxyurs-12-ene-2-O-beta-D-glucopyranoside

2R_1=H R_2=OGlc ; 11,21-dioxo-3beta,15alpha,24-trihydroxyurs-12-ene-24-O-beta-D-glucopyranoside

59



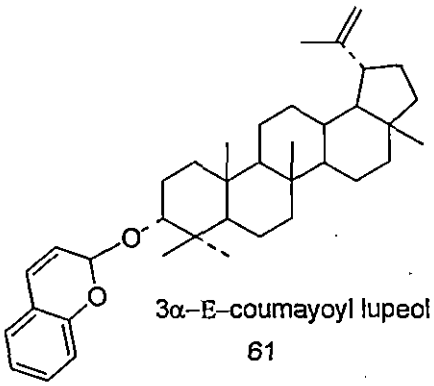
3 = 11,21-dioxo-3beta,15alpha,24-trihydroxyolean-12-ene-24-O-beta-D-glucopyranoside

(60)

Siddiqui *et al.*[40], isolated nine pentacyclic triterpenoids along with a coumarin from a fresh, undried and uncrushed spring leaves of *Plumeria obtuse*. They

characterized the new triterpenes obtusin and obtusilic acid as the 24-E and 27-Z-*p*-coumaric esters of the novel 3β,24-dihydroxyrus-12-en-28-oic acid and 3β,27-dihydroxyrus-12-en-30-oic acid respectively through chemical and spectral studies while the other eight compounds identified were known kaneroside, oleandrin, α-amyrin, neriucoumaric acid, isoneriucoumaric acid, alphitolic acid, oleanonic acid, methyl *p*-E-coumarate and scopoletin.

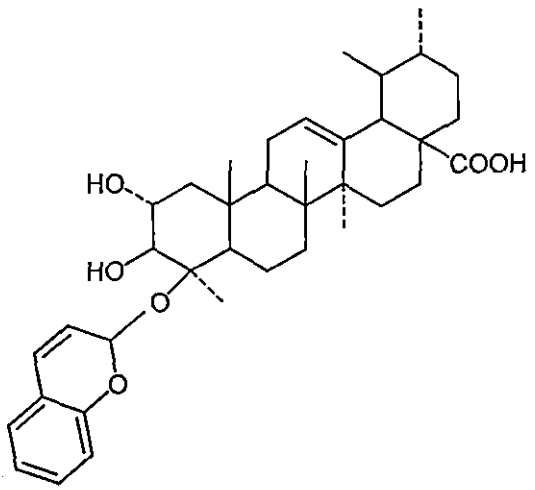
Karalai and Laphookhieo[41] isolated three new pentacyclic triterpenoid esters together with six known lupane-type triterpenoids from *Bruguiera cylindrica*. They elucidated the structures of the new compounds by spectroscopic methods and were characterized as 3α-E-coumaroyllupeol, 3α-Z-coumaroyllupeol and 3α-E-caffeoyltaraxerol.



3α–E–coumayoyl lupeol

61

Begum *et al.*[42] isolated three pentacyclic triterpenoids including one new and two known obtusinin and goreishic acid from the leaves of *Psidium guajava*. They characterized the new constituent as 2α-hydroxy-3β-*p*-E-coumaroyloxyurs-12, 18-dien-28-oic acid through 1 H-NMR and 13 C-NMR. They isolated compound guajavanoic acid first time from the genus *Psidium*.

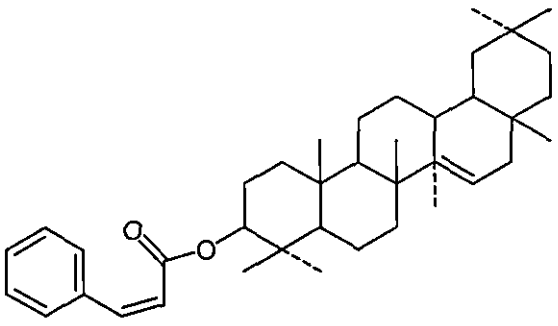


2α,3β-dihydroxy-24-p-E-coumaroyloxyurs-12-en-28-oic acid

62

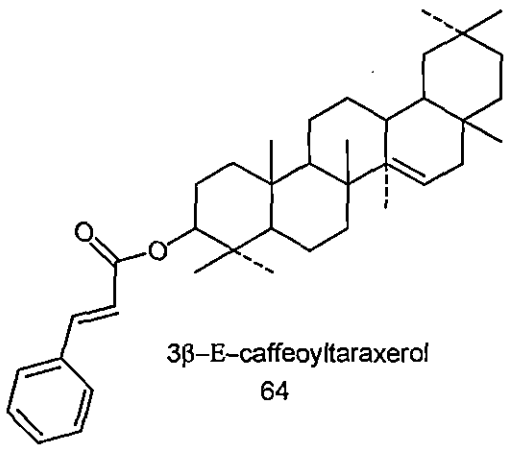
Larshini *et al.*[43], extracted 12 plants, selected on the basis of the folk-medicine reports and examined their anti bacterial effects against eight pathogenic bacteria. They found that the n-butanol extract of *Calotropis procera* flowers and the aqueous extract of *Eugenia caryophyllata* were the most effective against the bacteria they tested.

Laphookhieo *et al.*[44] isolated a new sesquiterpene and two new pentacyclic triterpenoid esters together with three known compounds from the fruits of *Rhizophora mucronta*. They elucidated the structures of the isolated compounds and characterized as 3-hydroxy-3,7,11-trimethyl-9-oxododeca-1,10-diene, 3β-E-caffeoyltaraxerol and 3β-Z-caffeoyltaraxerol.



3β–Z–caffeoyltaraxerol

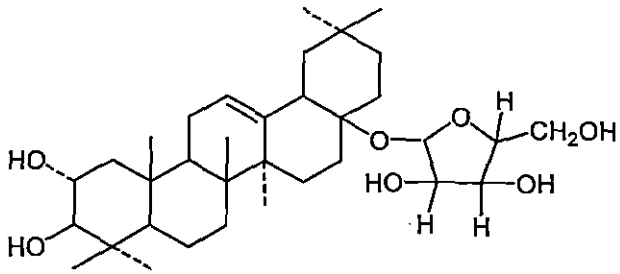
63



3β–E–caffeoyltaraxerol
64

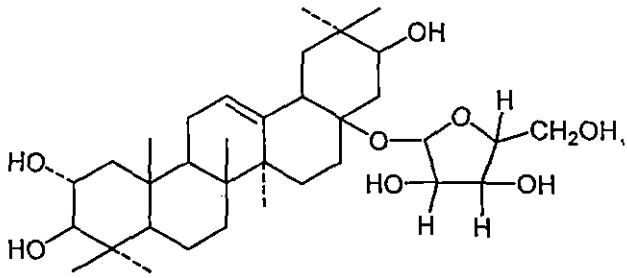
Ibrahim and Ali [45] isolated a long chain ketone, a pentacyclic triterpenoid coupled with fatty acid moiety, and an acyclic diterpenoid from the ethanol soluble part of *Nepeta crassifolia* collected from Kangavar, Iran. They elucidated the structures of all the metabolities with the aid of spectroscopic techniques, including 2D NMR experiments.

Tabopda *et al.* [46] isolated four new triterpene glucosides (**a-d**) using methanol as a solvent from the stem bark of *Terminalia superba*. The structures of the new compounds were established by spectroscopic method and characterized as 2-alpha,3-beta-dihydroxyolean-12-en-28-oic acid 28-O-beta-D-glucopyranoside (**a**), 2-alpha,3-beta,21 beta-trihydroxyolean-12-en-28-oic acid 28-O-beta-D-glucopyranoside (**b**), 2-alpha,3-beta,29-trihydroxyolean-12-en-28-oic acid 28-O-beta-D-glucopyranoside (**c**) and 2-alpha,3 -beta,23,27-tetrahydroxyolean-12-en-28-oic acid 28-O-beta-D-glucopyranoside (**d**) together with the known triterpene 2-alpha,3-beta,23-trihydroxyolean-12-en-28-oic acid (**e**). They investigated the antibacterial activity of **a-e** against two gram-positive bacteria (*Bacillus subtilis, Staphylococcus aureus*), and four Gram-negative (*Escherichia coli, Shigella flexenari, Pseudomonas aeruginosa, Salmonella typhi*) bacterial strains.



2α,3β-dihydroxyolean-12-en-28-oic acid-28-pyranoside

65



2α,3β,21β-trihydroxyolean-12-en-28-oic acid-28-pyranoside

66

2. Potential antimicrobial activities of triterpenoids and their derivatives

The reports presented by the earlier workers regarding the antimicrobial activity of various plant extracts were tested against different organisms. The observation (selective in manner) of the previous workers in concord with the present line of investigation have been presented in the following paragraphs.

Kumar *et al.*[47] carried out antimicrobial properties of a series of 61 medicinal plants belonging to 33 different families used in various infectious disorders at 1000 and 500 microg/ml concentration by agar dilution method against *Bacillus cereus, Bacillus pumilus, Bacillus subtilis, Bordetella bronchiseptica, Micrococcus luteus, Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa , Streptococcus faecali, Candida albicans, Aspergillus niger and Saccharomyces cerevisiae.* They found that 28 plant extracts showed activity against at least one of the test organisms used. The crude extracts of *Jatropha gossypifolia, Aristolochia indica, Lantana camara, Cassia fistula* containing triterpenoid as chemical constituent exhibited significant antimicrobial activity and

property that support the folkloric use in the treatment of as broad-spectrum antimicrobial agents.

Adamu *et al.*[48] carried out a survey of medicinal plants used locally in the treatment of various diseases in Bauchi State-Nigeria and total 84 medicinal plants were listed chiefly containing triterpenoids. They investigated the antimicrobial activity of the aqueous extracts of the plants and found that out of 84 plants, 75 exhibited antimicrobial activity against one or more of the test organisms at a concentration of 200 mg/ml. They found that the extracts showed potentially interesting activity against *Proteus mirabilis, Pseudomonas aeruginosa, Staphylococcus aureus* and *Escherichia coli.*

Angeh *et al.*[49] isolated four known triterpenoids, 1alpha,3beta-dihydroxy-12-oleanen-29-oic acid (a), 1-hydroxy-2-olean-30-oic acid (b), 3,30-dihydroxyl-12-oleanen-22-one (c), and 1,3,24-trihydroxyl-12-olean-29-oic acid (d) along with a new pentacyclic triterpenoids (1alpha,23-dihydroxy-12-oleanen-29-oic acid-3beta-O-2,4-di-acetyl-L-rhamnopyranoside)(e) through a bioassay-guided procedure from the leaves of *Combretum imberbe.* They found that all the isolated compounds had moderate (62 μg/ml) to strong (16 μg/ml) antimicrobial activity (MIC values) against *Staphylococcus aureus, Escherichia coli,* and compound a and e was most active. The results of their study gave credence to the ethnomedicinal use of *Combretum imberbe* and biological activity of the metabolites.

Mothana *et al.*[50] studied the antiproliferative activity against three human cancer cells, antimicrobial activity against antibiotic susceptible three Gram-positive, three Gram-negative bacterial and one fungal stains and three multiresistant Staphylococcus strains by the agar diffusion method and the determination of MIC against three Gram-positive bacteria with the broth micro-dilution assay, as well as for their antioxidant activity using the DPPH radical scavenging method of sixty four methanolic and aqueous extracts of thirty Yemeni plants used in traditional medicine. They found that 12 plants showed growth inhibitory effect against all cancer cells with IC50 values< 50 μg/ml, 9 plants showed pronounced antimicrobial activity against Gram-positive bacteria among them multiresistant bacteria with inhibition zones>15 mm and MIC values < 500 μg./ml.

Shai *et al.*[51] isolated four compounds lupeol, betulinic acid, ursolic acid and 2-alpha-hydroxyursolic acid from the leaves of *Curtisia dentata*. They studied the antibacterial and antifungal activity using broth microdilution assay and bioautography method and found that betulinic acid and ursolic acid were antimicrobial.

Mansouri[52] found new antibacterial agents from ethanolic extracts of ten plants mainly containing triterpenoids as chemical constituent. The agents were effective against *Staphyllococcus aureus*. Several samples (489 samples) of *S.aureus* were isolated from healthy carriers (nose and throat) or clinical samples. Out of 489 isolates 98.6% were sensitive to trimethoprim-sulfamethoxazole. The extracted compounds from the plants were screened for antibacterial activity. *Menta vividis L.* was active against some isolates and inhibited the growth of 48.7% of the isolates.

Samy and Ignacimuthu[53] reported the antifungal activity of crude drug from the tree bark of *Terminalia arjuna* containing triterpenoid which was tested against bacteria using the hole-plate diffusion method with concentrations of 5-25 mg/mL. The effective results of bacteria were confirmed by the dilution method (1.25-2.0 mg/ml) in MIC. The results were supported by phytochemical analysis. The specific activity against pathogenic bacterium, *Bacillus subtilis* and *Staphylococcus aureus* showed the traditional usage of bark of *T. arjuna.*

Al-Hussaini *et al.*[54] extracted 20 plant leaves and screened their inhibitory effect against the rice blast pathogen. They reported that triterpenoids containing plant *Prosopis juliflora* followed by *Zizyphus jujube* significantly inhibited the mycelial growth and biomass as well as toxin production and spore germination under laboratory conditions.

Mehmood *et al.*[55] studied the antimicrobial potential of some Indian medicinal plants containing triterpenoids and their formulations. They tested twenty five different formulations based on five alcoholic extracts against several pathogenic micro-organisms. They observed that ten formulations showed higher potency compared to their constituents and good synergistic activity leading to significant reduction in the MIC values.

Ragasa *et al.*[56] extracted the air dried leaves of *Vitex negundo* which afforded vitexilactone and casticin by silica gel chromatography. Their structures were elucidated by extensive 1D and 2D NMR spectroscopy. They studied their activity and found to inhibit the growth of the fungi: *Candida albicans* and *Aspergillus niger* and the bacteria: *Staphylococcus aureus* and *Pseudomonas aeruginasa.*

Ettebong and Nwafor[57] studied the antimicrobial activities of n-hexane, chloroform, ethyl acetate and methanol extract of *Carpolobia lutea* root which were used as a folk medicine in southern Nigeria against four typed cultures of bacteria namely, *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus subtilis* and two clinical strains of fungi, namely *Candida albicans* and *Tinea capitis* using agar well diffusion method. They reported that the ethyl acetate extract gave the widest zone of inhibition (21.0 mm) followed by chloroform when tested against *E.coli.* They also reported that none of the extracts showed any inhibitory effect against *Pseudomonas aeruginosa* and the fungal strains of *Candida albicans* and *Tinea capitis* and the most potent of these extracts was chloroform extract with Minimum Inhibitory Concentration (MIC) of 25 mg/ml for bacteria. The Phytochemical screening of the root of *C. lutea* revealed the presence of saponins, anthraquinones, flavonoids, cardiac glycosides, simple sugar and terpenes.

Horiuchi *et al.*[58] isolated the effective compound and identified it as oleanolic acid, a triterpenoid from *Salvia officinalis* (Sage) leaves and tested antimicrobial activity against vancomycin-resistant enterococci (VRE). They also tested the antimicrobial activity of similar triterpenoids, ursolic acid, uvaol, betulinic acid and betulin and found that ursolic acid also showed antimicrobial activity against VRE. The minimum inhibitory concentrations (MICs) of oleanolic acid and ursolic acid were 8 and 4 μg/ml, respectively and these two compounds also showed antimicrobial activity against *Streptococcus pneumoniae* and methicillin-resistant *Staphylococcus aureus* (MRSA). They found that these compounds also showed antibacterial activity against VRE at least for 48 h when added at concentrations that were two-times higher than their MICs.

Khan *et al.*[59] isolated amblyone, a triterpenoid from *Amorphophallus campanulatus* and studied *in vitro* antibacterial, antifungal and cytotoxic activities using disc diffusion technique and minimum inhibitory concentration was determined using serial dilution technique. They observed large zones of inhibition in disc diffusion antibacterial screening against four Gram-positive bacteria (*Bacillus subtilis, Bacillus megaterium, Staphylococcus aureus* and *Streptococcus pyogenes*) and six Gram-negative bacteria (*Escherichia coli, Shigella dysenteriae, Shigella sonnei, Shigella flexneri, Pseudomonas aeruginosa* and *Salmonella typhi*) and the MIC values against these bacteria ranged from 8 to 64 µg/ml. In antifungal screening, the compound showed small inhibition zones against *Aspergillus flavus, Aspergillus niger, Rhizopus aryzae. Candida albicans* was resistant against the compound.

Khan *et al.*[60] extracted crude from the leaves, stem bark, stem heart wood, root bark and root heart wood of *Euroschinus papuanus* and isolated fractions on partitioning with petrol, dichloromethane (D), ethyl acetate (E) and butanol (B) and studied antibacterial and antifungal activity. They observed that E fractions of the stem heart wood, D of root bark and E of root heart wood demonstrated excellent antibacterial activity and B fractions of leaves; stem heartwood and root bark demonstrated antifungal activity.

Ramesh *et al.*[61] tested the antimicrobial efficiency of aqueous, methanol, chloroform and hexane extracts of *Swertia corymbosa* containing triterpenoids and noticed maximum inhibitory activity against *Staphylococcus aureus* and *Salmonella typhi.*

Ahmad and Beg[62] extracted 45 Indian plants traditionally used in medicine using ethanol as a solvent and studied their antimicrobial activity against certain drug-resistant bacteria and a yeast *Candida albicans.* They showed that out of the 45 plants 40 plant extracts contained terpenoids and were effective antimicrobial activity against one

Smith *et al.*[63] performed a screening of eight plants from Belize for antibacterial activity. They reported that six plants showed activity against the four organisms tested. Both inoculum density and medium type played important roles in assay sensitivity.

Akinpelu[64] observed that *Anacardium occidentale* bark containing triterpenoids 60 percent methanolic extract exhibited antimicrobial activity against 13 out of 15 bacterial isolates at a concentration of 20 mg/ml.

Audu *et al.*[65], extracted components from *Ziziphus abyssinica* (root bark) containing triterpenoids using methanol, diethyl ether and cold water as solvent. They studied their activity on *Candida albicans*, *Escherichia coli*, *Salmonella* spp. and *Staphylococcus aureus* at different concentrations and found that all these components inhibited the growth of microbes.

Ramesh *et al.*[66] isolated Friedelin, epi-Friedelin, n-Octacosanol, α–Amyrin, Sitosterol, Sitosterol-3-D-glucopyranoside and luteoforol from *Bridelia crenulata* Roxb. The aqueous and methanolic extracts and their fractions were tested against ten human pathogenic bacteria and four fungal strains. They observed that inhibitory activities were maximum in the chloroform-methanol (1:1) fraction of the methanolic extract against *E.coli*, *K.pneumoniae* and *P.aeruginasa*, which were responsible for the pathogenesis of urinary tract infection. The above study provided scientific evidence for the efficacy of the use of *Bridelia crenulata* extracts.

Murillo-Alvarez *et al.*[67] extracted compounds from plants used in the traditional medicine of Baja California sur (Mexico) using ethanol as a solvent. They tested antimicrobial activities of the isolated compounds. The antimicrobial activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Streptococcus faecalis*, *Candida albicans* and *Escherichia coli* were determined. *Aristolochia monticola*, *A.brevipes*, *Hymenoclea sp.* were found to be the most active.

Habtemariam and Macpherson[68] investigated the cytotoxic and antibacterial activity of an ethanol extract of leaves of a herbal drug *Eupatorium perfoliatum* containing triterpenoids. They observed that the extract showed a potent cytotoxicity and weak antibacterial activity against gram positive test organisms *Staphylococcus aureus* and *Bacillus megaterium*.

Lall *et al.*[69] observed that the water and acetone extracts of roots of *Euclea natalensis* containing triterpenoids inhibited the growth of *Bacillus cerus*, *Bacillus*

pumilus, Bacillus subtilis, Micrococcus kristinae and *Staphylococcus aureus* at concentration ranging between 0.1 and 6.0 mg/ml. They found that the water extract did not exert any inhibitory action on Gram-negative bacteria while the acetone extract showed inhibitory activity at a concentration of 5.0 mg/ml against all the Gram-negative bacteria investigated. The antibacterial activity of acetone extract was also investigated by a direct bioassay on TLC plates against *S. aureus*

Alves *et al.*[70] evaluated the antimicrobial, antifungal and antiadherent activity of *P :sidium guajava* containing triterpenoids on oral biofilm microorganisms and oral *candidiasis in vitro*. They found that the extracts were shown to be effective in inhibiting the growth of bacteria of the oral biofilm and fungi of oral *candidiasis*.

Duraipandiyan *et al.*[71] studied the antimicrobial activity of 18 ethnomedicinal plant mostly containing triterpenoids collected from Palni hills of southern western ghats against nine bacterial strains (*Bacillus subtilis, Staphylococcus aureus, Staphylococcus epidermidis, Enterococcus faecalis, Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa, Ervinia sp, Proteus vulgaris*) and one fungal strain(*Candida albicans*) using paper disc diffusion method. They reported that out of 18 plants, 10 plants exhibited antimicrobial activity against one or more of the tested microorganisms at three different concentrations of 1.25, 2.5and 5 mg/disc. The study evaluated the antimicrobial activity of some ethnomedicinal plants used in folkloric medicine.

Bonjar[72] studied the antibacterial activities of the 45 species of 29 plant families used in the traditional medicine by Iranian people against *Bacillus cereus, Bacillus pumilus, Bordetella bronchiseptica, Escherichia coli, Klebsiella pneumoniae Micrococcus luteus, Pseudomonas aeruginosa, Pseudomonas fluorescens, Serratia marcescens, Staphylococcus aureus, Staphylococcus epidermidis*. He found that no plant showed activity against *Serratia marcescens* but *Bordetella bronchiseptica* were the most susceptible species. All extracts showed the same activity even after 18 months.

Saleh *et al.*[73] isolated the known triterpenoids lantic acid, camarinic acid and lantanilic acid from *Lantana camara* (L) cultivated in Egypt and carried out the antibacterial activity of lantic acid using bioautography assays for Gram-positive and Gram-negative bacteria. They found that lantic acid possess strong antibacterial activity

against *Escherichia coli* and *Bacillus cereus* in which 0.08 and 0.1 µg were the minimum inhibition doses compared to 0.05 and 0.005 µg for chloramphenicol. Their results showed that lantic acid had broad spectrum antibacterial activity.

Mathabe *et al.*[74] isolated four known compounds from the stem bark of *Spirostachys africana* using ethanol as a solvent which was used traditionally for the treatment of diarrhoea and dysentery in Limpopo province of South Africa. The isolated compounds were, two triterpenoids, compound 1 [d-Friedoolean-14-en-oic acid (3-acetyl aleuritolic acid)] and compound 2 (lupeol), and two diterpenes, compound 3[ent-2, 6alpha-hydroxy-norbeyer-1,4,15-trien-3-one (diosphenol2)] and compound 4 (ent-3beta-hydroxy-beyer-15-ene-2-one). They tested the antibacterial activity using micro dilution method and found that compound 1, exhibited MIC of 50 microg/ml against *Staphylococcus aureus, Salmonella typhi, Vibrio cholera* and *Escherichia coli* compound 2 was not active against all tested microorganisms at 200 microg/ml.

Escalante *et al.*[75] isolated three monodesmosidic triterpenoid saponins from the butanolic extract of *Phytolacca tetramera* and established their structures. They reported that the three saponins belong to the olean-type triterpenoid saponins, with 28,30 dicarboxylic groups and an olefinic double bond on C-12. They observed that phytolaccosides B and E showed antifungal activities against a panel of human pathogenic opportunistic fungi but phytolaccoside F did not show any activity. The most sensitive fungus was *Trichophyton mentagrophytes.*

Yasunaka *et al.*[76] studied the antibacterial activity of the thirty two extracts from 22 Mexican medicinal plants of 15 different families mostly containing triterpenoids against *Escherichia coli* and *Staphylococcus aureus.* They reported that seventeen plants showed antibacterial activity.All the 17 extracts except one showed higher activity against *Staphylococcus aureus* than *Escherichia coli.*

Khan *et al.*[77] extracted the leaves, seeds, stem and root barks, stem, root and heart-woods of *Michelia champaca* containing terpenoids using methanol, petrol, dichloromethane, ethyl acetate, butanol as a solvent. They observed that different fractions exhibited antibacterial activity. They also observed that fractionation drastically enhanced the level of activity particularly in the fractions of the stem bark,

dichloromethane fraction of the root bark and some fractions of the leaves.Stem and root bark extracts showed activity against some of moulds. They found that liriodenine was the active constituent of the root bark.

Aqueveque *et al.*[78] isolated a new biologically active triterpenoid favolon B from fermentation broths of *Mycena* sp. Strain 96180. They found that favolon B showed antifungal activities against *Botrytis cinerea, Mucor miehei, Paecilomyces variotii* and *Penicillium notatum.* Flavon B did not have any activities against bacteria and yeast.

Kirmizigul *et al.*[79] reported antimicrobial and antifungal activities of the MeOH extract from the flowers of *Cephalaria transsylvanica* and three triterpenic acid glycosides, transsylvanoside A-C by MeOH using an agar-disc diffusion method. They observed that both the MeOH extract and the glycosides possess antimicrobial and antifungal activities against *Staphylococcus aureus, Escherichia coli, Proteus vulgaris, Pseudomonas aeruginosa, Corynebacterium xerosis, Klebsiella pneumonia, Candida utilis, Kluyveromyces fragilis, Aspergillus oryzae* and *Aspergillus flavus* respectively.

Sudharmini and Ashalatha[80] isolated triterpenoids from *Myxopyrum smilacifolium* leaf and found the presence of ursolic acid (0.175mg/l). They reported that the triterpenoids showed antimicrobial activity against gram positive bacteria. *Candida albicans* was resistant against the compound.

Mbwambo *et al.*[81] extracted compounds from stem bark, wood and whole roots of *Ternimalia brownie* containing triterpenoids using solvents of increasing polarity, namely, pet ether, dichloromethane, dichloromethane: methanol (1:1), methanol and aqua, respectively and the extracts were tested for antifungal and antibacterial activity. They observed that the extracts of the stem bark, wood and whole roots of *T. brownii* exhibited antibacterial activity against several bacteria (*Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia, Salmonella typhi* and *Bacillus anthracis)* and the fungi, (*Candida albicans* and *Cryptococcus neoformans).* They found that aqueous extracts exhibited the strongest activity against both bacteria and fungi.

Jacinda *et al.*[82] extracted large quantities of pentacyclic triterpenoids, saponin collectively known as Centelloids. These terpenoids include Asiaticoside, Centelloside,

madecassoside, Brahmode etc. *In Planta,* the *Centella* triterpenoids can be regarded as phytoanticipins due to the antimicrobial activity and protective role against attempted pathogen infection.

Sharma *et al.*[83] reported that ingestion of *Lantana camara* foliage by grazing animalscauses intrahepatic cholestatis and amociated liver damage. The hepatotoxins were pentacyclic triterpenoides called Lantadenes.A number of biological activities have been associated with various parts of *Lantana* in folk medicine.Roots of Lantana plants were rich in oleanolic acid, a heteroprotective triterpenoid.Pentacyclic triterpenoids were the focus of attention for drug research for anti-cancer, anti-AIDS, anti inflammatory and anti microbial activities.

Rajenderan *et al.*[84] isolated pentacyclic triterpenoids, namely Ursolic acid, 2-hydroxy ursolic acid, (Asiatic acid, glycerol-1,2-dilinolexyl-3-o-b-D-galactopyronose from the methanolic extract of leaves of *M. malabathricum*.This plant was selected as one of the most promising source of antimicrobial agents.It showed anti-viral,cytotoxic, anti-oxidant, anti-cancer activity,anti-hypertensive activity,anti-inflammatory and anti-pyretic properties.

Chaudhury *et al.*[85] showed that the phytochemicals betulinic acid (a), wogonin (b) and oroxindin (c) isolated from the aerial parts of *Bacopa monnieri* and *Holmskioldia sanguinea* showed significant antifungal activity against the two fungi *Alternaria alternata* and *Fusarium fusiformis*. Inhibition of root growth germination of wheat seeds was observed for all three compounds which showed 100% inhibition at 10 micro g/mL. Compounds (a) and (b) showed potent inhibition of *Alternaria alternata* compared with oroxindin at a concentration of 4 micro g/mL, whereas compound (b) was an effective inhibitor of both fungi.

Ghosh *et al.*[86] isolated of two triterpenoids betulinic acid and lupeol from the leaf extract of *Psidium guajava* and their potential antimicrobial and phytotoxic activities. All the structures of the isolated compounds were confirmed by spectral (IR, NMR) analysis and by comparison with the literature reports available.

Betulinic acid is a naturally occurring pentacyclic triterpenoid and has been shown to exhibit a variety of biological activities including inhibition of human

immunodeficiency virus (HIV), antibacterial, antimalarial, antiinflammatory, antithelmintic and antioxidant properties reported by Yogeeswari.*et al.*[87].

Shafi *et al.*[88] reported that the Leaves of two plants, *a* terpenoid fraction of both plants showed significant antifungal activity against *F. equiseti, C. gloeosporioides* and *A. alternata* when tested by spore germination method. MIC values of terpenoid fraction of *D. stramonium* extract were 0.05mg/ml against *F. equiseti,* 0.1mg/ml against *C. gloeosporioides* and 0.5mg/ml against *A. alternata* when tested by disc diffusion method. Similarly, the MIC of *C. mucronata* leaf extract against *F. equiseti* was found to be 0.01 mg /ml. The MIC value of *D. stramonium* leaf extract (terpenoid fraction) was 0.5mg/ml against *A. alternata.*

One plant extract (*Datura*) containing triterpenoids was subjected to column separation. The separated fractions were tested against *F. equiseti* and *C. gloeosporioides* following spore germination bioassay. Potential fractions (fraction 6-20 & fraction 26-35) were also tested by disc diffusion bioassay. Combined column fractions (6- 20) could inhibit the growth of *C. gloeosporioides* at a concentration of 0.2mg/ml. Another column fraction (26–35) could check the spore germination of the *F. equiseti* at a concentration of 0.08mg/ml reported by Barre *et al.*[89].

Ahmed *et al.*[90] screened antifungal compounds (terpenoids) from the roots of the wild carrot, *Daucus carota* L. ssp. *carota* (Apiaceae) and it was found to contain a range of antifungal activity against *Fusarium oxysporum* and *Aspergillus niger.*

Barrero *et al.*[91] reported that *a* wide spectrum of secondary metabolites such as phenols, flavonoids, quinones, tannins, essential oils, terpenoids, alkaloids, saponins and sterols which showed antimicrobial activity. The compounds were isolated from higher plants. They investigated antifungal efficacy of sesquiterpene lactones isolated from the six *Centaurea* species (*C. bombycina* Boiss ex D.C., *C. granatensis* Boiss, *C. monticola* Boiss, *C. incana* Desf., *C. maroccana* Ball. and *C. sulphurea* Willd.) against the fungus *Cunninghamella echinulata.* They reported that the two compounds costunolide and dehydrocostunolide, were responsible for the antifungal activity.

Scher *et al.*[92] prepared a dichloromethane and a methanol extract of the liverwort *Bazzania trilobata* (L.) S.F. Gray (Lepidoziaceae) and showed their antifungal

activity against some phytopathogenic fungi (*Botrytis cinerea*, *Cladosporium cucumerinum*, *Phythophthora infestans*, *Pyricularia oryzae* and *Septoria tritici)* due to the presence of terpenoids. From the extracts they isolated six antifungal sesquiterpenes: 5-and 7-hydroxycalamenene, drimenol, drimenal, viridiflorol, gymnomitrol and chloroisopiagiochin.

Digrak *et al.*[93] showed that compound extracted from *Terminalia arjuna* containing triterpenoids was found to be effective against *F.equiseti* and other two pathogens.The leaf extract of *Clerodendrum viscosum* could completely check the radial growth of the test fungi.

A mixture of loliolide 1 (>85%) and paniculatadiol 2 (<15%) was obtained from the ethyl acetate leaf extract of *Pterocarpus indicus* by silica gel chromatography, while the air-dried flowers afforded lupeol 3 and phytol esters 4. The structures of **1-4** were determined by NMR spectroscopy. Antimicrobial tests on a mixture of **1** and **2** indicated that it has moderate activity against *Candida albicans* and low activity against *Pseudomonas aeruginosa*, *Escherichia coli*, and *Aspergillus niger*. It was found inactive against *Staphylococcus aureus, Bacillus subtilis*, and *Trichophyton mentagrophytes* reported by Ragasa *et al.*[94]

Parveen *et al.*[95] reported that the methanolic extract of the leaves of *Peltophorum vogelianum* (Caesalpiniaceae) afforded a new phytoconstituent, 2-methoxy-4,5-dihydroxy-1(7,8-dihydroxyethylene)-8-β-D-glucuropyranoside named as peltophorumyl-β-D-glucuropyranoside (**e**), along with four known phytoconstituents, 1-pentatriacontanol (**a**), friedelin (**b**), β-sitosterol (**c**) and β-sitosterol-β-D-glucopyranoside (**d**). The structures were established on the basis of chemical and physical evidence (IR, ^{1}H-NMR, ^{13}C-NMR, DEPT, HSQC, HMBC and MS). Moreover, Compound **5** showed significant antimicrobial activity.

Ramesh *et al.*[96] made various extracts of the leaves of *Begonia malabarica* Lam. (Begoniaceae). From the extracts of six known compounds, (viz. friedelin, epi-friedelinol, beta-sitosterol, luteolin, quercetin and beta-sitosterol-3-beta-D-glucopyranoside) were isolated. The aqueous and organic solvent extracts were also tested against ten human pathogenic bacteria and four fungal strains by the agar-well

diffusion method. All the extracts were devoid of antifungal activity against the tested fungi.

Duraipandiyan *et al.*[97] designed an experiment to evaluate the antifungal activity of *Azima tetracantha* extracts and isolated compound (friedelin) against fungi. They fractionated the extract through silica gel column. The antifungal activity of *Azima tetracantha* crude extracts and isolated compound (friedelin) were evaluated using the micro dilution method. Hexane extract showed some antifungal activity. The lowest MIC against *Trichophyton rubrum* was 62.5 µg/ml and the MIC against *Curvularia lunata* was 62.5 µg/ml.

Shing *et al.*[98] reported that the hexane extract of *Heliotropium marifolium* yielded a mixture of triterpenoids: β-sitosterol, stigmasterol, β-amyrin, friedelan-3β-ol (epifriedelenol), cycloartenone, β-amyrin acetate, friedelin and epifriedenyl acetate. Their Isolated triterpenoid and reference antibiotics (gentamycin/mycostatin) were tested against selected pathogenic bacteria and fungi, e.g. *Escherichia coli, Staphylococcus aureus, Aspergillus niger* and *Penicillium chrysogenum*. The inhibition zone (IZ) and the activity index (AI) of isolated compounds were recorded. They reported that epifriedenyl acetate (IZ = 17; AI = 1.06) was most active.

Joseph *et al.*[99] reported that the 50% methylene chloride in hexane fraction (column fraction of the extract of leaves of *Ficcus racemosa*) showed antifungal activity.. The extract inhibited the growth of several plant pathogens (*Curvularia sp, Colletotrichum gloeosporioides, Alternaria sp* and *Fusarium sp).*

Siddiqui *et al.*[100] isolated a new pentacyclic triterpene, oleanderol and the known betulin, betulinic acid, ursolic acid and oleanolic acid from the leaves of *Nerium oleander*.The structure elucidation of oleanderol and identification of betulin,betulinic acid, ursolic acid and oleanolic acid had been carried out through chemical and spec tral studies.They had antimicrobial properties also.

From the above literature, the present work were designed.Several compounds were isolated and derieved. All the compounds were tested for antimicrobial activities. In the following chapters the process of the isolation, preparation of derivatives and their bioassay have been discussed in details.

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■ CHAPTER - II

Title: Extraction and characterization of Lupeol from *Xanthoxylum budrunga*, preparation of its derivatives and antimicrobial activity of each of them

Plants provide food and other life supporting commodities and are very important for the survival of man and other organisms. They protect our environment and maintain nature. The evidence of man's dependency on plant for his survival can be demonstrated by palaeo-ethnobotanical finding from prehistoric archeological sites [1]. The wild medicinal and aromatic plants are widely distributed in the mountains and forests of the eastern India. They are valuable sources of medicine for domestic and commercial purpose [2]. Although the rural people utilize wild plants for their livelihood, the scientists have recently realized the importance of such plants in rural economy.

The present chapter deals with isolation, characterization and production of four antimicrobial derivatives of naturally occurring triterpenoids of the plant *Xanthoxylum budrunga*. *Xanthoxylum budrunga* belongs to the family Rutaceae and distributed throughtout the world although substantial occurance have been reported in Zimbabwe, eastern Africa and southern Africa. In India it occurs in the Himalayan region of northeast India [3]. The plant is very common in sub-Himalayan forests.

Medicinally and commercially *Xanthoxylum budrunga* is a very useful tree. Wood is used for bundles, walking sticks and fishing rods. It is suitable for heavy construction, heavy flooring, interior trim, vechile bodies, furniture, cabinet works, main props, ladders, sporting goods, agricultural implements, toys, musical instruments, boxes, crates. [4].

The stem bark is used to treat cough, cold, boils, pleurisy, toothache and snakebite. The prickles are applied to infected wounds. The bark is used to treat chest pain and as a poultice to heal sores.Roots are used to treat mouth ulcers, sore throat and as a tonic [5] .The prickle bearing protuberances on the bole are also used by the children as toys.

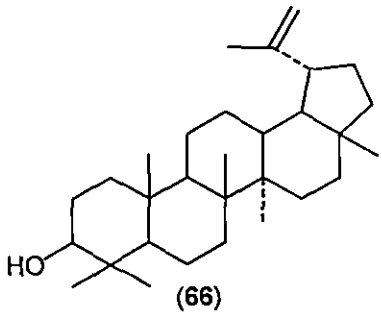
Morphologically, this plant is a dioecious, medium sized tree up to 30m tall, bole usually straight and cylindrical up to 60cm in diameter, conspicuous woody, prickled bearing protuberances, bark pale gray in younger tree to dark brown in older ones, twigs glabrous and armed with long prickles upto 5mm. Leaves alternate, imparipinnately compound with(5-7-13 phyllotaxy), leaflet up to 30cm long, stipules absent, rachis chamelled above, leaflet opposite, sessile, slightly asymmetrically oblong to lanceolate, connate and slightly notched at apex, margin finely toothed, glabrous pinnately veined with numerous lateral veins. Inflorescence terminal, panicle with flowers in clusters. Flowers unisexual, regular, small, pedicel 1-1.5cm long; sepal nearly free, 0.5mm, petals elliptical 2.5mm long; greenish yellow in color; male flower with 4 stamens; ovary rudimentary;female flowers with superior, globose ovary and short style. Fruits a globose follicle, glandular pitted, dehiscent; one seeded, black and shiny [6].

Xanthoxylum budrunga has a good medicinal as well as commercial prospect. The medicinal properties deserve more attention because of the claimed antimicrobial activities which have been confirmed by pharmacological research [7].

Section 1: Extraction and characterization of pentacyclic triterpenoids (Lupeol) from *Xanthoxylum budrunga.*

1.1 Extraction from the bark of *Xanthoxylum budrunga*: Isolation of lupeol (compound A)

Dried and powdered trunk bark of *Xanthoxylum budrunga* (2 kg) was extracted with chloroform in Soxhlet apparatus for 20 hours. Chloroform was distilled off and the gummy residue (1 kg) was taken up in ether (1.5 lt). The ether solution was washed with 10% aqueous sodium hydroxide solution (3x300 ml). The aqueous alkaline layer was thoroughly shaken with ether to remove any neutral material that might be present. The ether portion was washed with water till neutral and then dried by using Na_2SO_4. Ether was removed when a gummy residue (5g) of Lupeol was obtained. This residue dissolved in petroleum ether (30ml) was placed over a column of silica gel (30 g) developed with petroleum ether and was eluted with following solvents (table 2.1).



(66)

Lupeol

Table 2.1: Examination of different fractions obtained from column chromatographic separation

Eluent	Fractions 50ml each	Residue on evaporation	Melting point
Petroleum ether	1-4	nil	-
Petroleum ether:ethylacetate(1:4)	5-8	nil	-
Petroleum ether:ethylacetate(3:2)	9-19	solid	211°-213°C

Further elution with more polar solvent did not afford any solid material

1.2. Chemical characterization of fractions:

Fractions 9-19 (Table2.1) were combined and crystallized from a mixture of chloroform and methanol. The compound was coded as 'A' and was characterized. Melting point (m.p.) was determined as 215° C. On the basis of IR, TLC and melting point the compound resembled as lupeol. Finally, the compound was confirmed as lupeol when compared with authentic sample of lupeol.

1.3: Preparation of Bromo derivatives of Lupeol:

Following steps have been carried out to prepare the bromo derivatives:

1.3.1: Hydrogenation of lupeol: Preparation of lupanol

Lupeol (coded as compound A) (5g) dissolved in a mixture of ethylacetate and acetic acid (80 ml each) was shaken in an atmosphere of hydrogen in presence of PtO_2 catalyst (0.2g) for three hours until absorption of hydrogen ceased. Ethylacetate was

removed by distillation and the solution was diluted with water whereby a white solid (4.5g) separated out which was collected by filtration. Crystalisation from a mixture of chloroform and methanol furnished colourless plates of lupanol and characterized. Melting point was determind as 204^0 C, $[\alpha]_D$ +15°. On the basis of IR, TLC and melting point the compound resembled as lupanol. Finally, the compound was confirmed as lupanol when compared with authentic sample of lupanol (co-tlc, mixed m.p *etc.*).

1.3.2 Jone's oxidation of lupanol: preparation of lupanone

To a solution of lupanol (4g) in pure acetone (400 mL), Jone's reagent was added drop wise with shaking until a faint orange colour persisted. The mixture was kept at room temperature for 1 hour, dilute with water and extracted with ether. The ether layer was washed thoroughly with water and dried by using Na_2SO_4. The ether was evaporated. The residue (3.5g) dissolved in petroleum ether was chromatographed over a column of silica gel (24 g). The chromatogram was developed with petroleum ether and then eluted with the following solvents (Table-2.2).

Table 2.2: Examination of different fractions obtained from column chromatographic separation

Eluent	Fractions 50ml each	Residue on evaporation	Melting point
Petroleum ether	1-4	nil	-
Petroleumether:ethylacetate(1:4)	5-8	solid	208^0-210^0C

Further elution with more polar solvent did not afford any solid material

1.3.3: Characterization of fractions:

Fractions 5-8 (Table2.2) were combined and crystallized from a mixture of chloroform and methanol. The crystallization furnished colourless needles of lupanone and characterized. Melting point (m.p.) was determined as 208^0 C. On the basis of IR, TLC and melting point the compound resembled as lupanone. Finally, the compound was confirmed as lupanone when compared with authentic sample of lupanone (co-tlc, mixed

m.p *etc.*). The characters of colourless needle shaped crystals were: m.p. 208^{0}C, [α] $_D^+$15 [Lit 67 m.p. 210^0 $(\alpha)_D^+$ 16.2]

Analysis report of the compound was compared in the following table along with some characters of the isolated compound.

Analysis report	%C	%H
Present compound	84.11	11.82
Calculated for $C_{30}H_{50}O$ (authentic sample)	84.52	11.74

1.3.4.: Treatment of lupanone with N-bromo succinimide: formation of 2,2–dibromo lupanone (compound B) and 2α-bromolupanone (compound C):

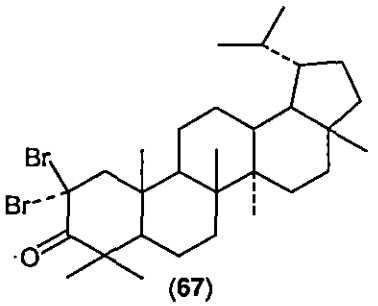
A solution of lupanone (3g) in chloroform (150ml) was mixed with dimethyl sulphoxide (75ml). N-bromosuccinimide (3.5g) was then added to it in small lots in order to keep the temperature of the reaction mixture below 25^{0}C and the mixture kept in dark for 10 days. It was extracted with chloroform and the extract washed several times with water, dried by using Na_2SO_4 and solvent removed under reduced pressure. The residue (2.8g) was chromatographed over a column of silica gel (18 g). The chromatogram was developed with petroleum ether and eluted with the following solvents (Table 2.3).

Table 2.3: Examination of different fractions obtained from column chromatographic separation

Eluent	Fractions 50ml each	Residue on evaporation	Melting point
Petroleum ether	1	oil	-
Petroleum ether	2-7	solid	208^0-209^{0}C
Petroleum ether:ethylacetate(4:1)	8-14	solid	221^0-222^{0}C

Further elution with more polar solvent did not afford any solid material

1.3.5:.Examination of fractions 2-7(Table2.3): Isolation of 2,2-dibromo lupanone (compound B) and characterization:



(67)
2,2-dibromo lupanone

The fractions 2-7 (Table2.3) showed homogeneity on TLC plate. Hence these were combined (1.1g) and crystallized from of mixture of chloroform and methanol to afford needle shaped crystals of 2,2-dibromolupanone coded as compound 'B' and characterized. Melting point (m.p.) was determined as 210^0C-211^0C and was identified as 2,2 dibromo lupanone.

Analysis report of the compound was compared in the following table along with some characters of the isolated compound.

Analysis report	%C	%H
Present compound	61.47	13.81
Calculated for $C_{30}H_{48}OBr_2$(authentic sample)	61.43	13.33

UV: λ_{MeOH} 222nm (ε = 7928)
Max 312nm (ε = 27)
Nujol
IR: ν_{max} 1722 cm-1(CO)
CD: λ_{CHCl3} 239 nm (θ = + 4590.18)
Max 320 nm (θ = - 8977.85)

1H NMR ($CDCl_3$): 0.77, 0.94, 0.97, 1.90, 1.24 (5s, 15H, 5t-CH3)
0.78, and 0.86 (2d, 6H, 2s-CH3, J = 7Hz)
3.13 and 3.64 (2d, 2H, 1-CH2, J = 16Hz) ppm

MASS: m/z at 586, 584,582 (M+), 567,569,571,
539,541,543,504,506,489,491,461,463,
426,425,409,285,283,274,231,206,205,
191,171,163,123(base peak)

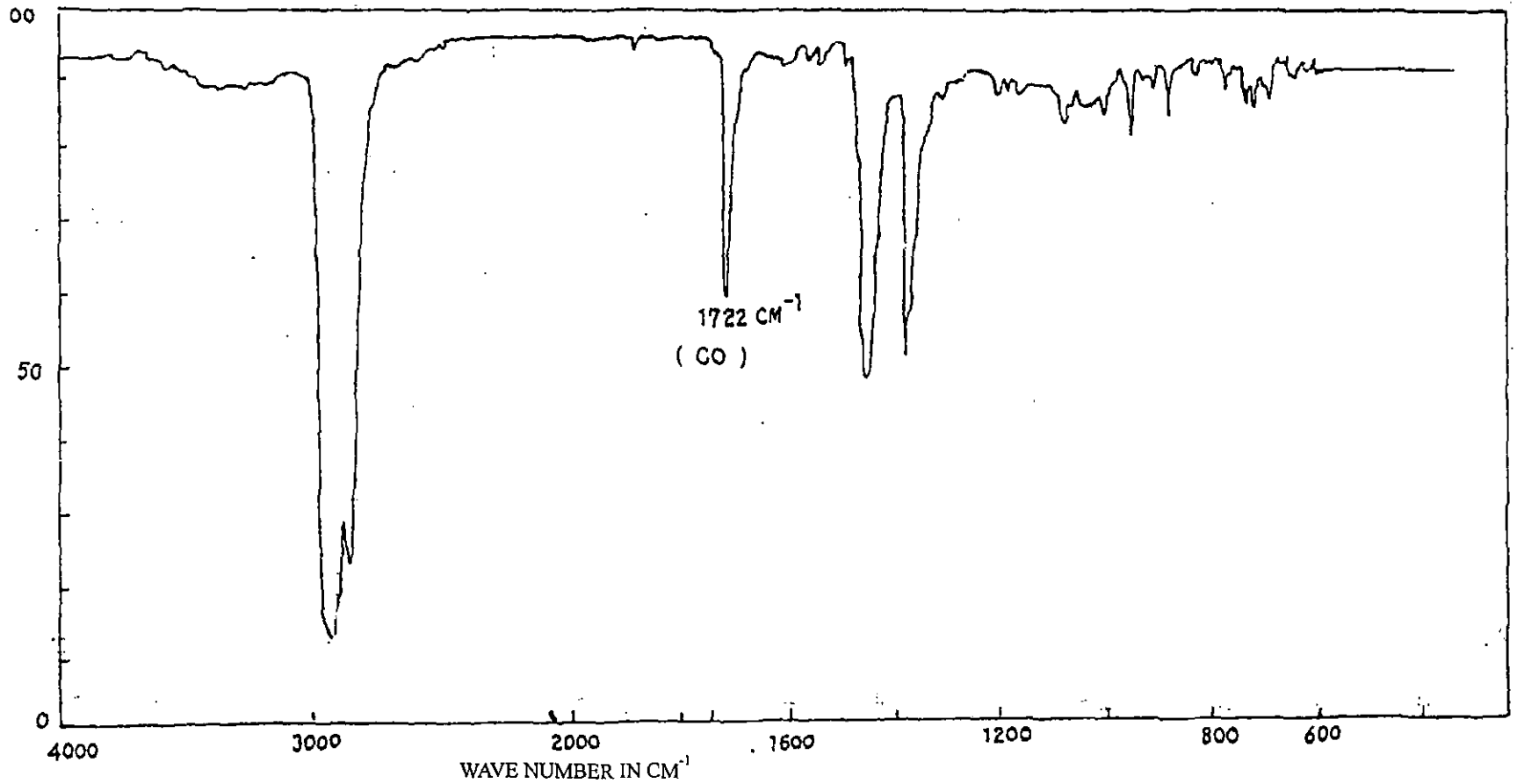


Fig. 1: IR Spectrum of 2,2-dibromolupanone (Compound B)

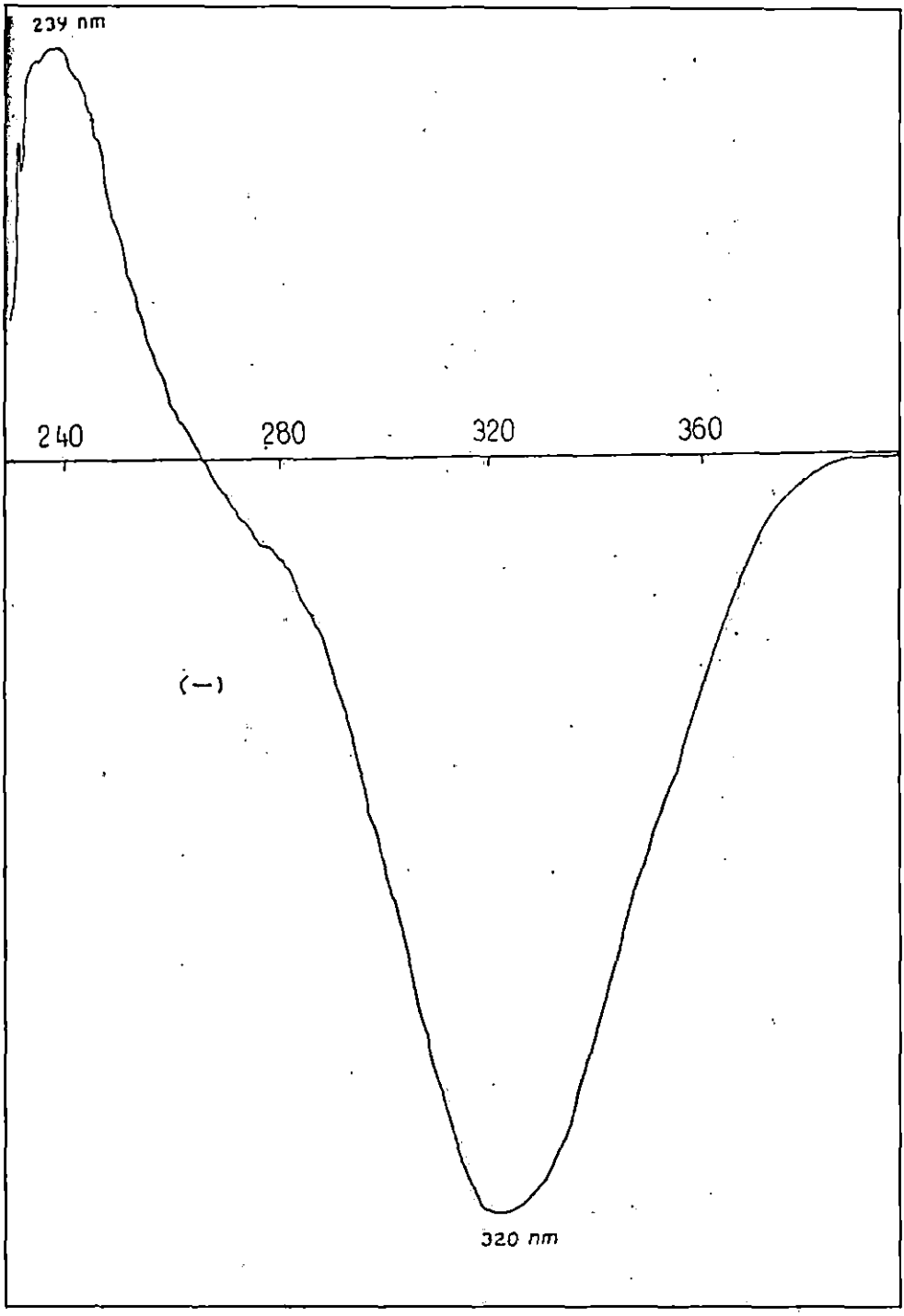


Fig. 2: CD Spectrum of 2,2-dibromolupanone (Compound B)

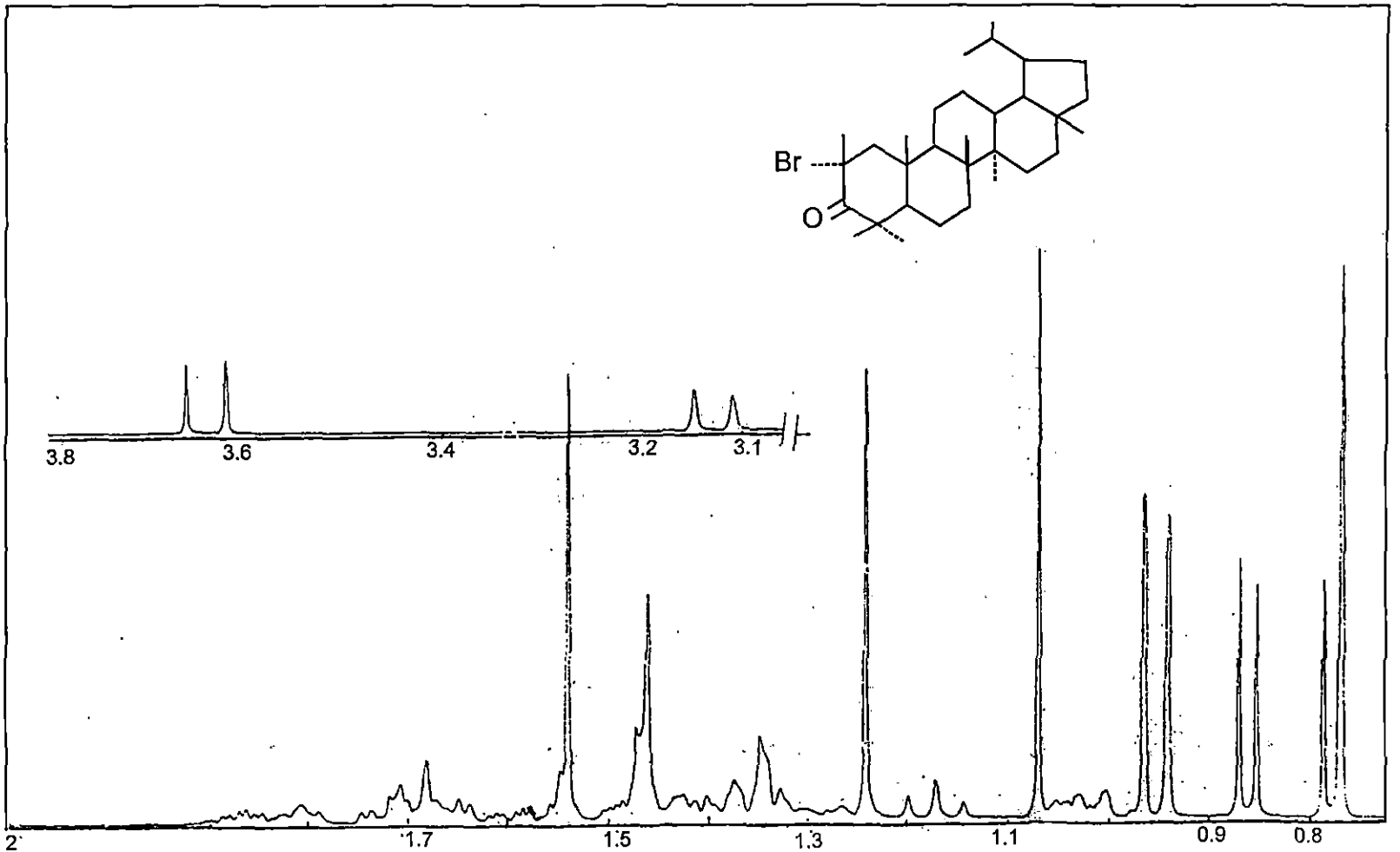


Fig. 3: NMR Spectrum of 2,2-dibromolupanone (Compound B)

$C_{30}H_{48}OB_2$

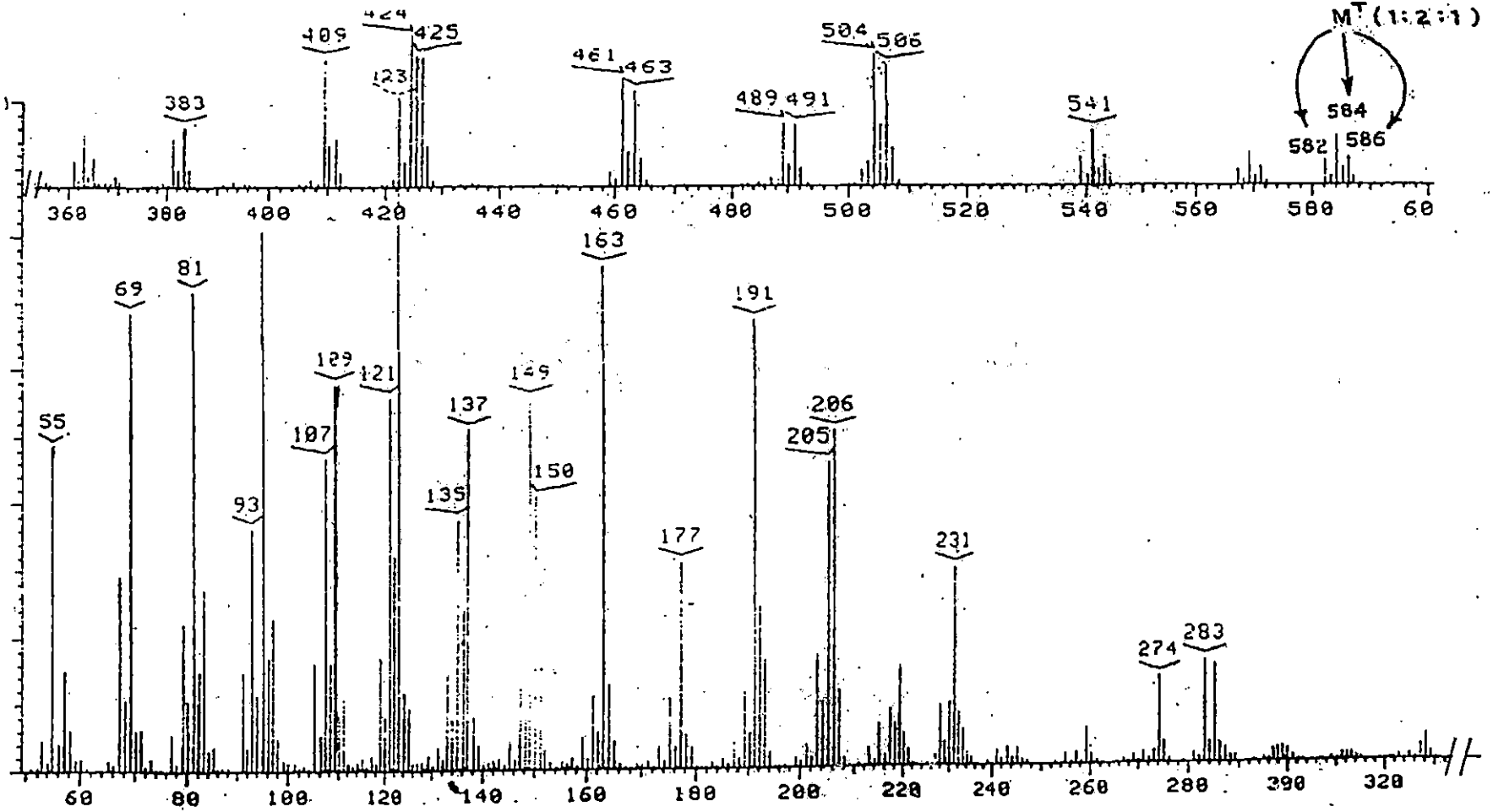
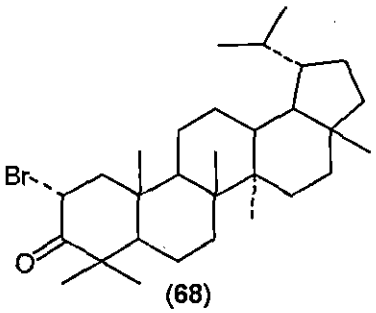


Fig. 4: Mass Spectrum of 2,2-dibromolupanone (Compound B)

1.3.6: Examination of fractions 8-14(Table2.3): Isolation of 2α-bromo lupanone (Compound C) and Characterisation:



(68)
2α-bromo lupanone

The fractions 8-14 (Table2.3) were combined (1.7g) and crystallized by chloroform –methanol mixture to afford amorphous white solid of 2α-bromolupanone coded as compound 'C'. Melting point (m.p.) was determined as 224^0C-225^0C and was identified as 2α-bromolupanone. It showed positive Belistein test for bromine.

Analysis report of the compound was compared in the following table along with some characters of the isolated compound.

Analysis report	%C	%H
Present compound	71.20	9.31
Calculated for $C_{30}H_{49}OBr$ (authentic sample)	71.15	9.68

UV: λ_{MeOH} 225 nm (ε = 7010)
Max 310 nm (ε = 42)

Nujol
IR: ν_{max} 1720 cm-1(CO)
CD: λ_{MeOH} 295 nm (θ = + 2620.82)
Max

1H NMR ($CDCl_3$): 0.77 (S, 6H, 2—CH3), 0.92, 1.10, 1.13, 1.2,
(4S, 12H, 4t—CH3)
0.76 and 0.85 (2d, 6H-2s-CH3, J = 7 Hz)
2.65 (dd, 1H, 1-c-He, J = 12 Hz and 6Hz)
2.67 (t, 1H, 1C-Ha, J =12Hz)
5.06 (dd, 1H, 2-CH, J =12 And 6Hz) ppm

MASS: m/z at 506, 504 (M+), 491,489,463,461,426,425,285, 283, 274, 206, 191, 163, 149, 123 (base peak)

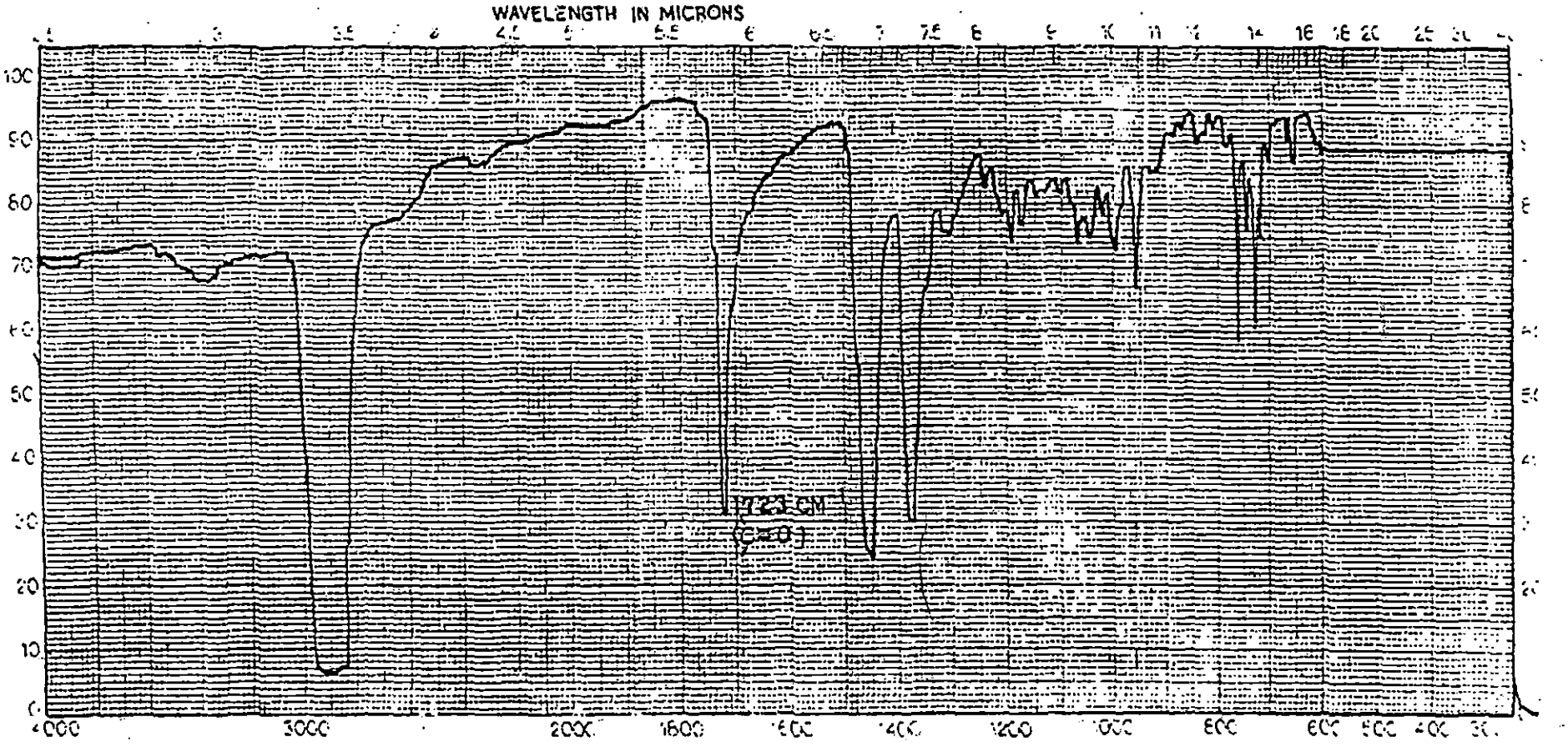


Fig. 5: IR Spectrum of 2α-bromolupanone (Compound C)

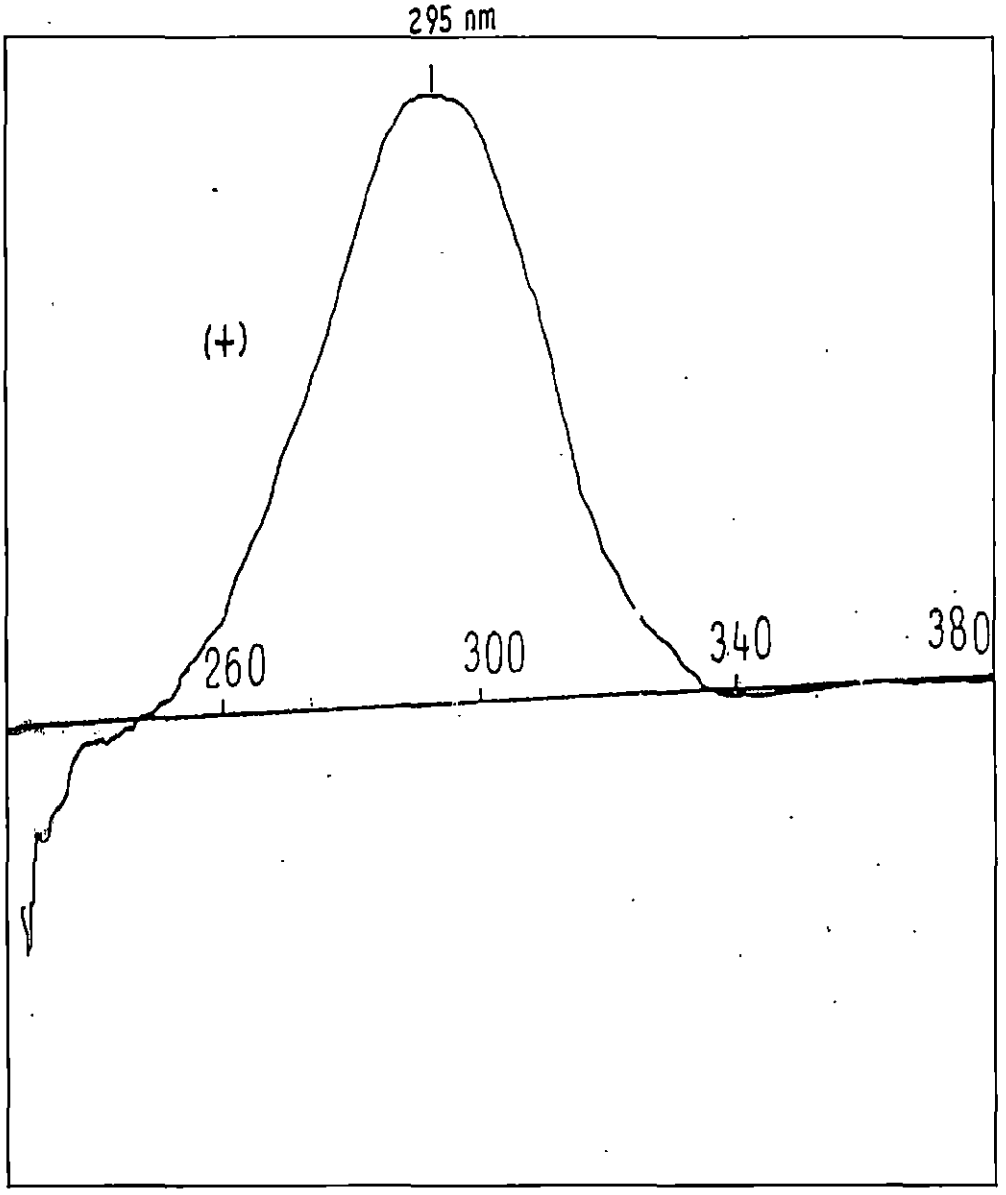


Fig. 6: CD Spectrum of 2α-bromolupanone (Compound C)

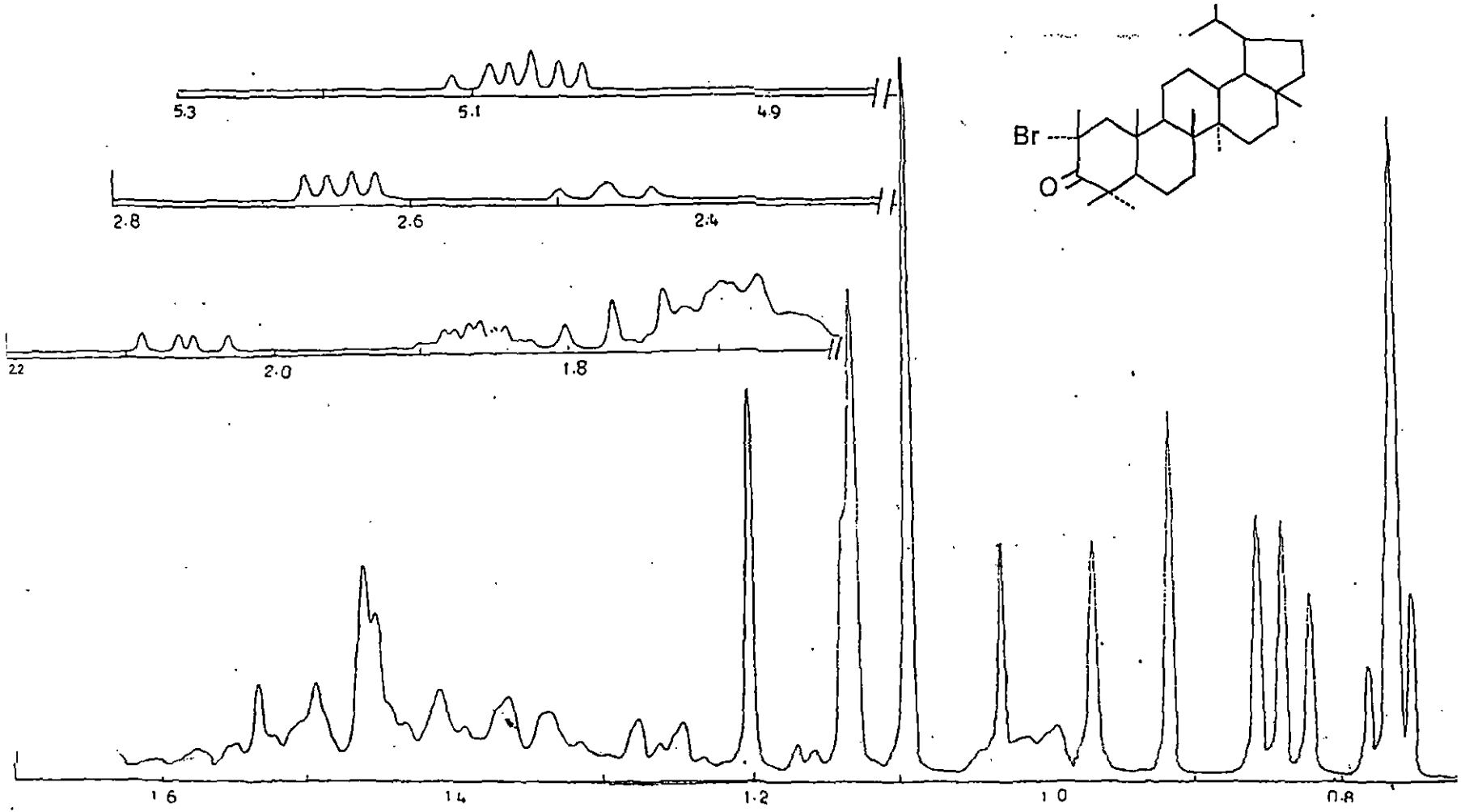


Fig. 7: ^{1}H NMR Spectrum of 2α-bromolupanone (Compound C)

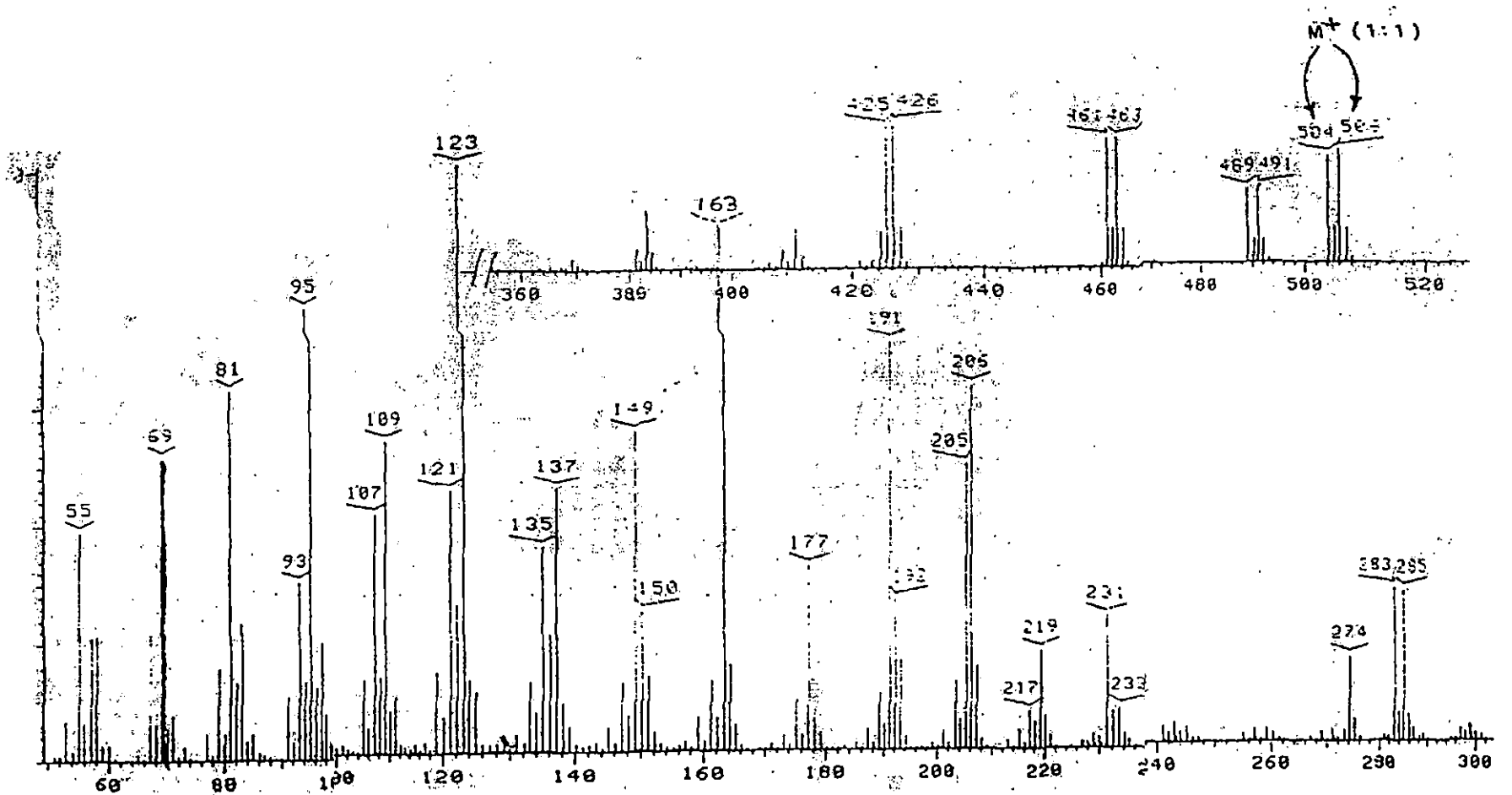
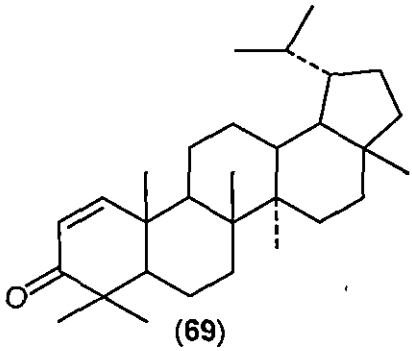


Fig. 8: Mass Spectrum of 2α-bromolupanone (Compound C)

1.3.7. Dehydro bromination of 2α-bromo lupanone with lithium bromide-N,N-dimethyl formamide: Isolation of lup-1(2)-en-3-one (compound D)



(69)
Lup-1(2)-en-3-one

Compound C (200g) (2α-bromo lupanone) was refluxed with distilled DMF (30 mL) and lithium bromide (300g) for six hours. The solid residue (180 mg) obtained after usual work up was chromatographed over a column of silica gel (10 g). The chromatogram was developed with petroleum ether and eluted with the following solvents (Table 2.4)

Table 2.4: Examination of different fractions obtained from column chromatographic separation

Eluent	Fractions 50ml each	Residue on evaporation	Melting point
Petroleum ether	1-3	nil	-
Petroleumether:ethylacetate(1:4)	4-12	solid	193^0-196^0C

Further elution with more polar solvent did not afford any solid material

Fractions 4-12 (Table2.4) showed homogeneity on TLC plate were combined (0.17g) and crystallized from chloroform-methanol mixture. The crystallization furnished colourless needle shaped crystals of lup-1(2)-en-3-one coded as compound D and characterized. Melting point of compound D was 198^0-199^0C and gave negative test for halogen.

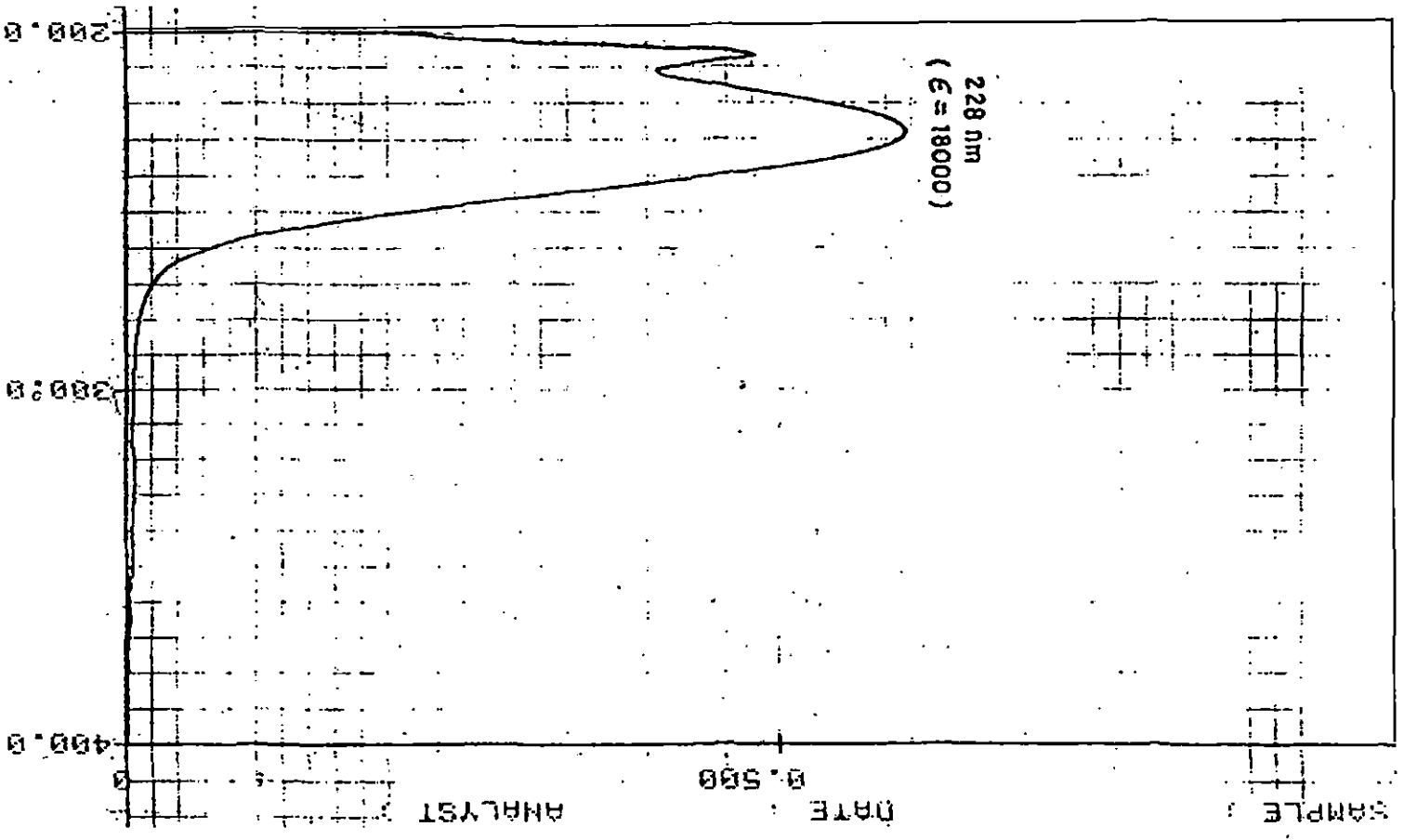


Fig. 9: UV Spectrum of 1up-1-(2)-en-3-one (Compound D)

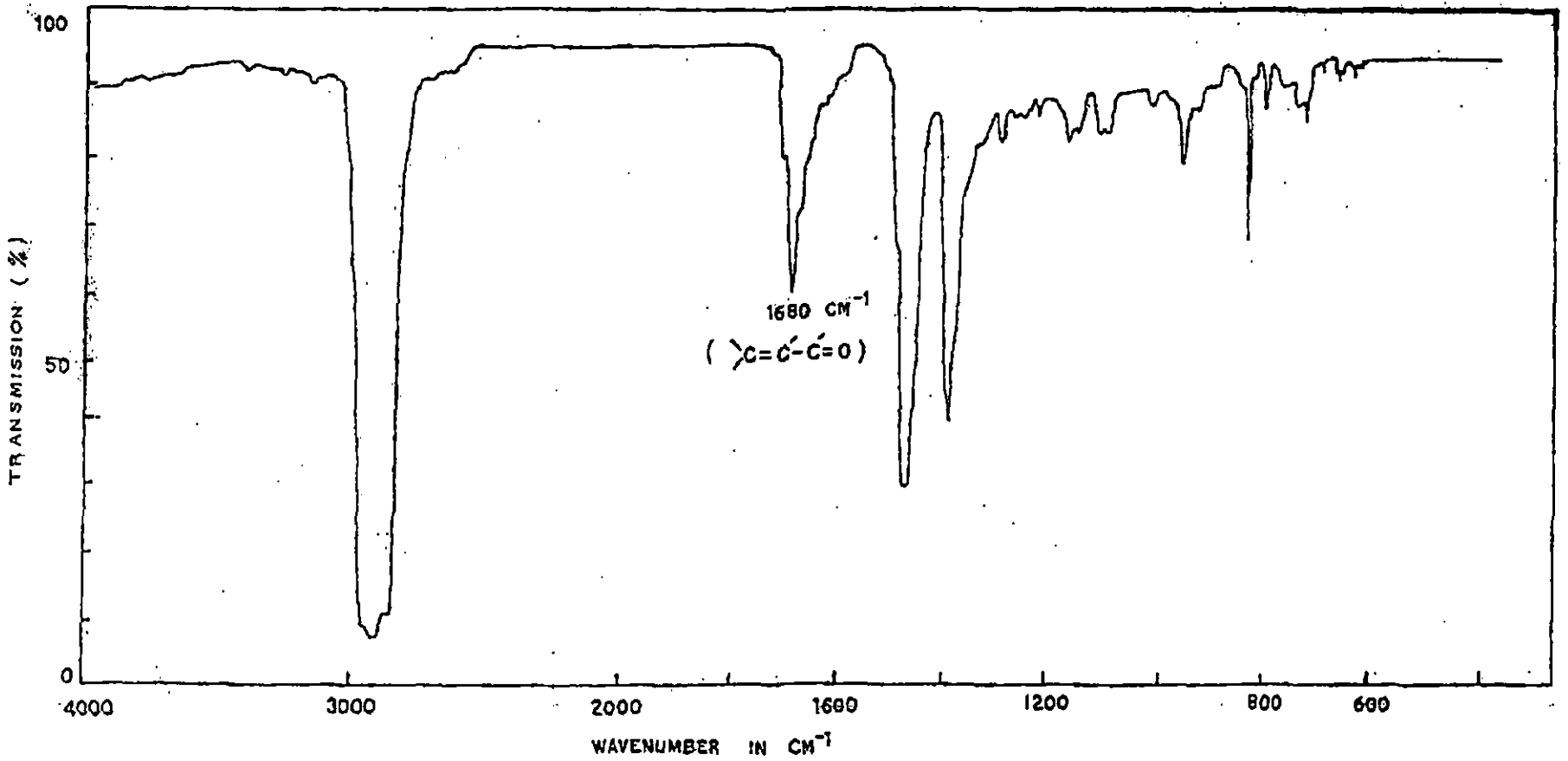


Fig. 10: IR Spectrum of 1up-1-(2)-en-3-one (Compound D)

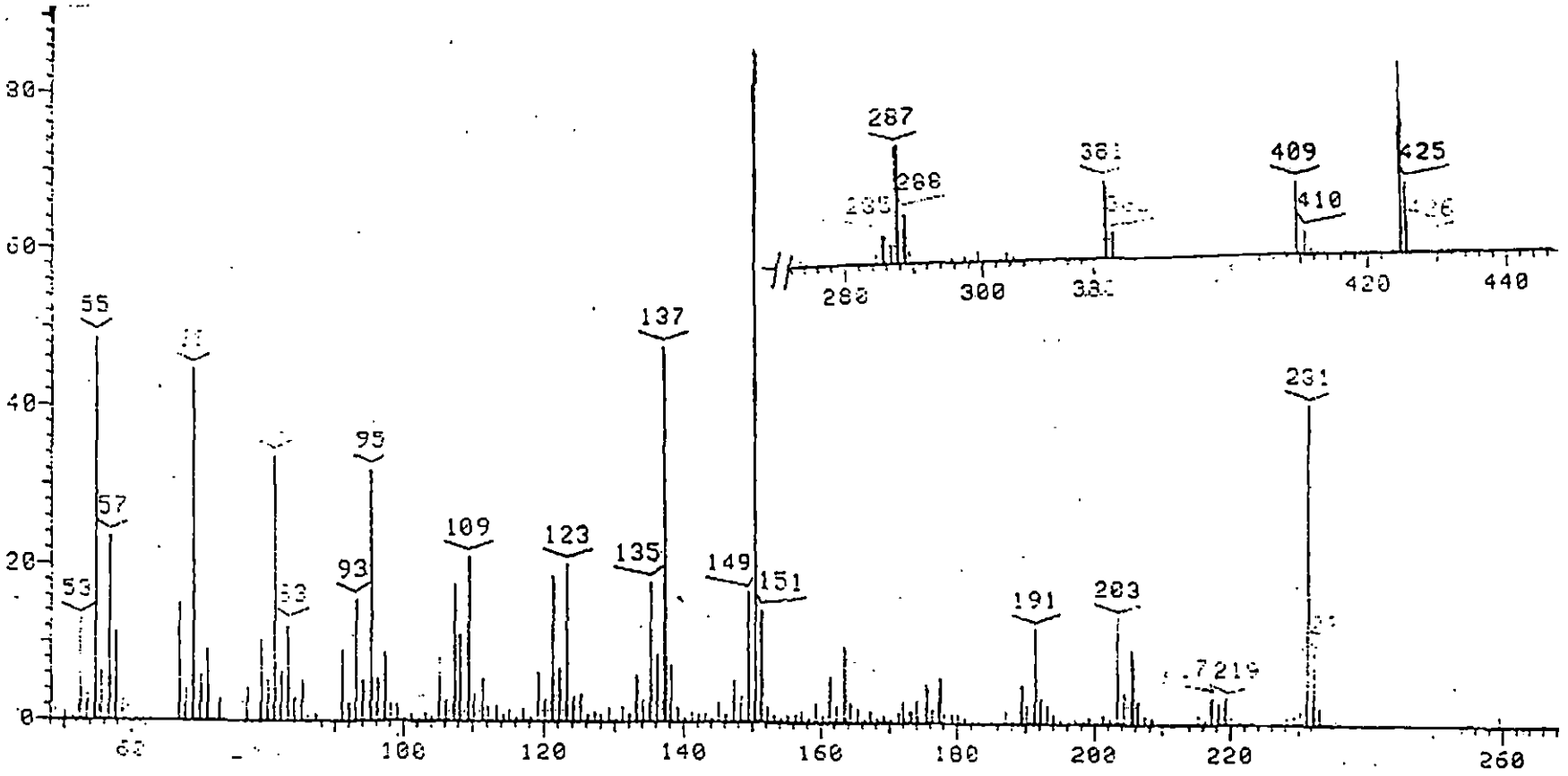


Fig. 11: Mass Spectrum of 1up-1-(2)-en-3-one (Compound D)

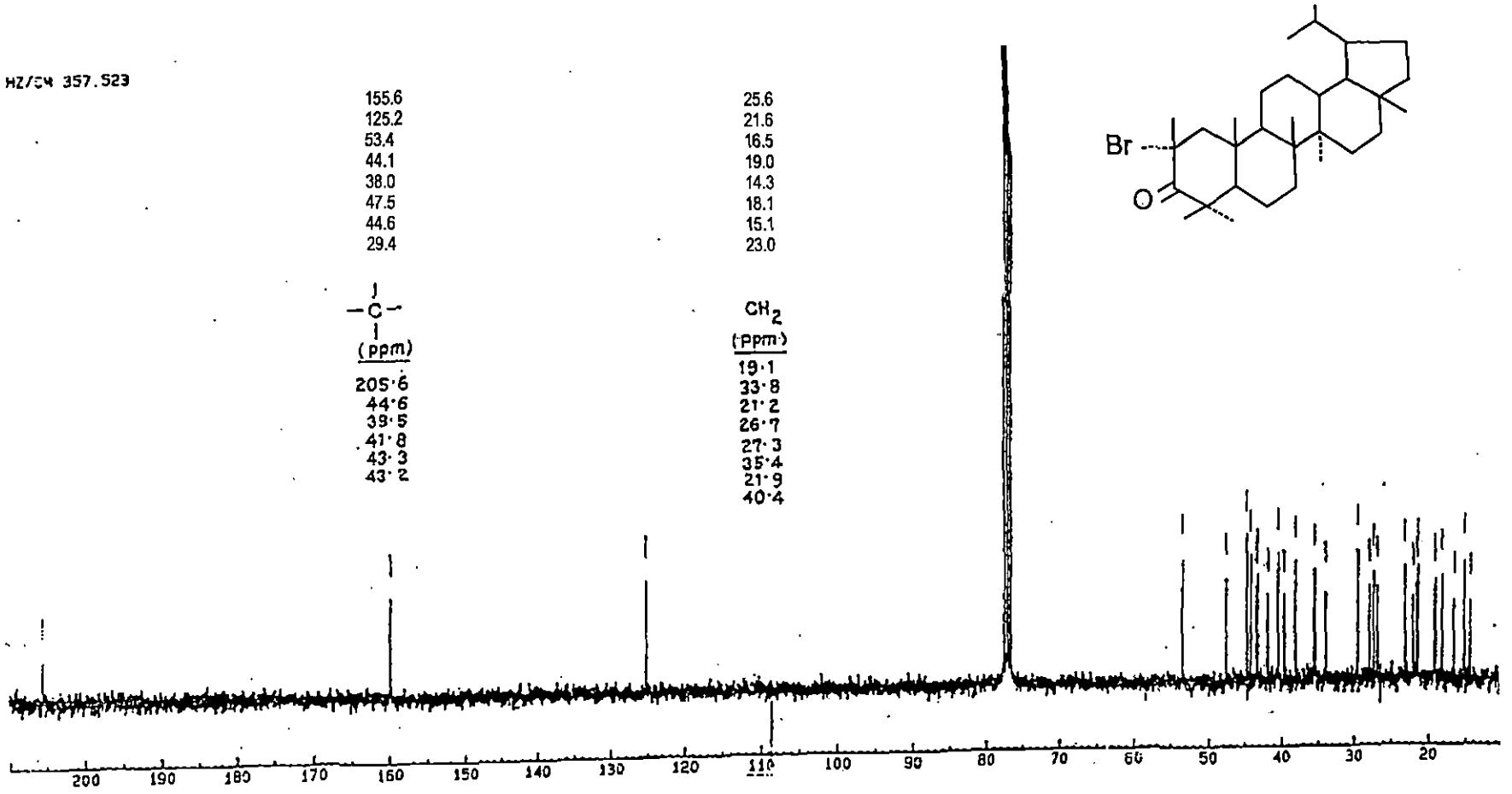


Fig. 12: ^{13}C NMR Spectrum of 1up-1-(2)-en-3-one (Compound D)

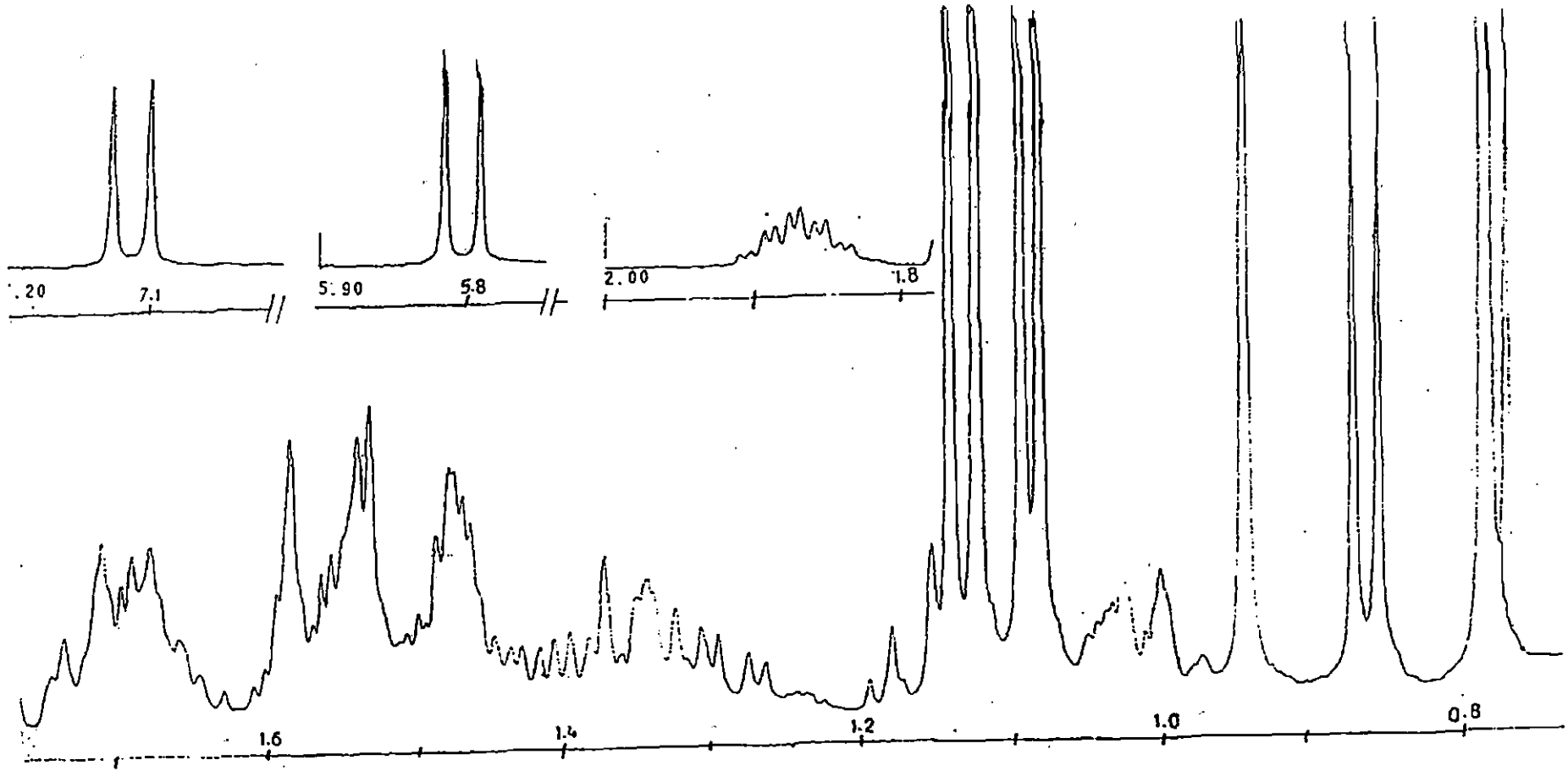


Fig. 13: ^{13}H NMR Spectrum of 1up-1-(2)-en-3-one (Compound D)

Analysis report of the compound was compared in the following table along with some characters of the isolated compound.

Analysis report	%C	%H
Present compound	84.79	11.21
Calculated for $C_{30}H_{48}O$ (authentic sample)	84.91	11.32

UV: λ_{MeOH}^{max} 228 nm (ε = 18,000)

IR: ν_{max}^{Nujol} *1680/cm (α,β-unsaturated ketone)*

1H NMR ($CDCl_3$): 0.79, 0.64, 1.07, 1.08, 1.11, 1.14t (6S, 18H 6t-ch3), 0.77 and 0.86 (2d, 6H, 2S-CH_3, J = 8 Hz), 5.80 and 7.11 (2d, 2H, H-C=C-H, J = 10 Hz) ppm

MASS: m/z at 424 (M^+), 381[M--$CH(CH_3)_2$]+, 288, 287, 231, 150 (base peak), 137, 95, 69, 55.

1.3.8. Preparation of lupan [2,3-C]-1′,2′,5′-oxadiazole (coded as compound E) from lupane 2,3dioxime:

Lupan [2,3-C]-1',2',5'-oxadiazole was prepared by cyclisation of 2,3-dioximino lupane (prepared by the treatment of 2,3-diketo lupan with hydroxyl amine-hydrochloride in ethanol) in dry DMF under microwave irradiation (100W, 100^0C) for 10 minutes. The compound obtain from the reaction was purified by repeated crystallization from chloroform-methanol mixture to obtain a compound of m.p 249^0-250^0C, analyzed for $C_{30}H_{48}ON_2$, which was corroborated by mass spectral analysis that showed the molecular ion at m/z 452. The other ions appeared at m/z 437 $[M\text{-}CH_3]^+$, 409 $[M\text{-}CH(CH_3)2]^+$ (BASE PEAK) and 367, 271, 259, 245, 231, 206, 191, 163, 149, 123, 121, 109, 95, 81 and 55. IRspectrum of the compound showed peaks at 1620 cm^{-1} (-C=N-O)and 890 cm^{-1} for heterocyclic ring. It showed UV absorption maximum at 223 nm (ε = 5169) for disubstitiuted furazan derivative. Interestingly in the NMR spectrum different methyl group signals got separated which may be due to the presence of heterocyclic ring attached with ring. It showed the presence of six tertiary methyls of which four of them

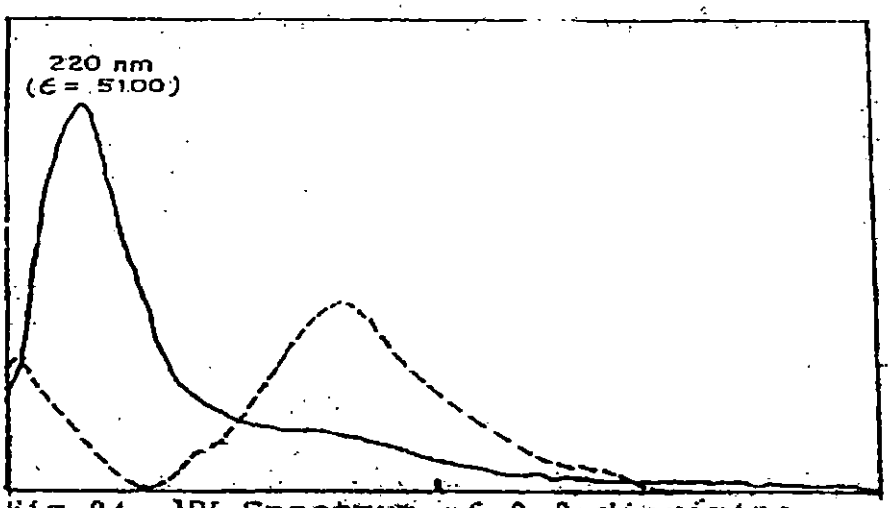


Fig.94 UV Spectrum of 2,3-dioximino-lupane(32)

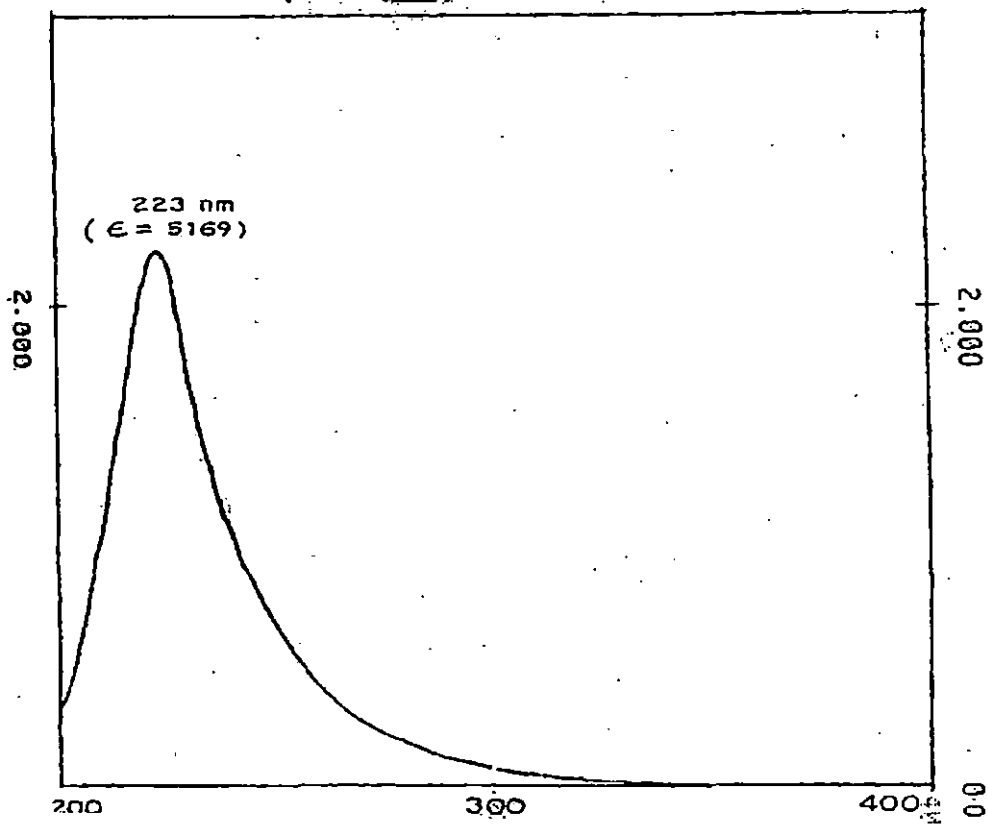


Fig. 14: UV Spectrum of lupan [2,3-C]-1′ 2′,5′-oxadiazole (Compound E)

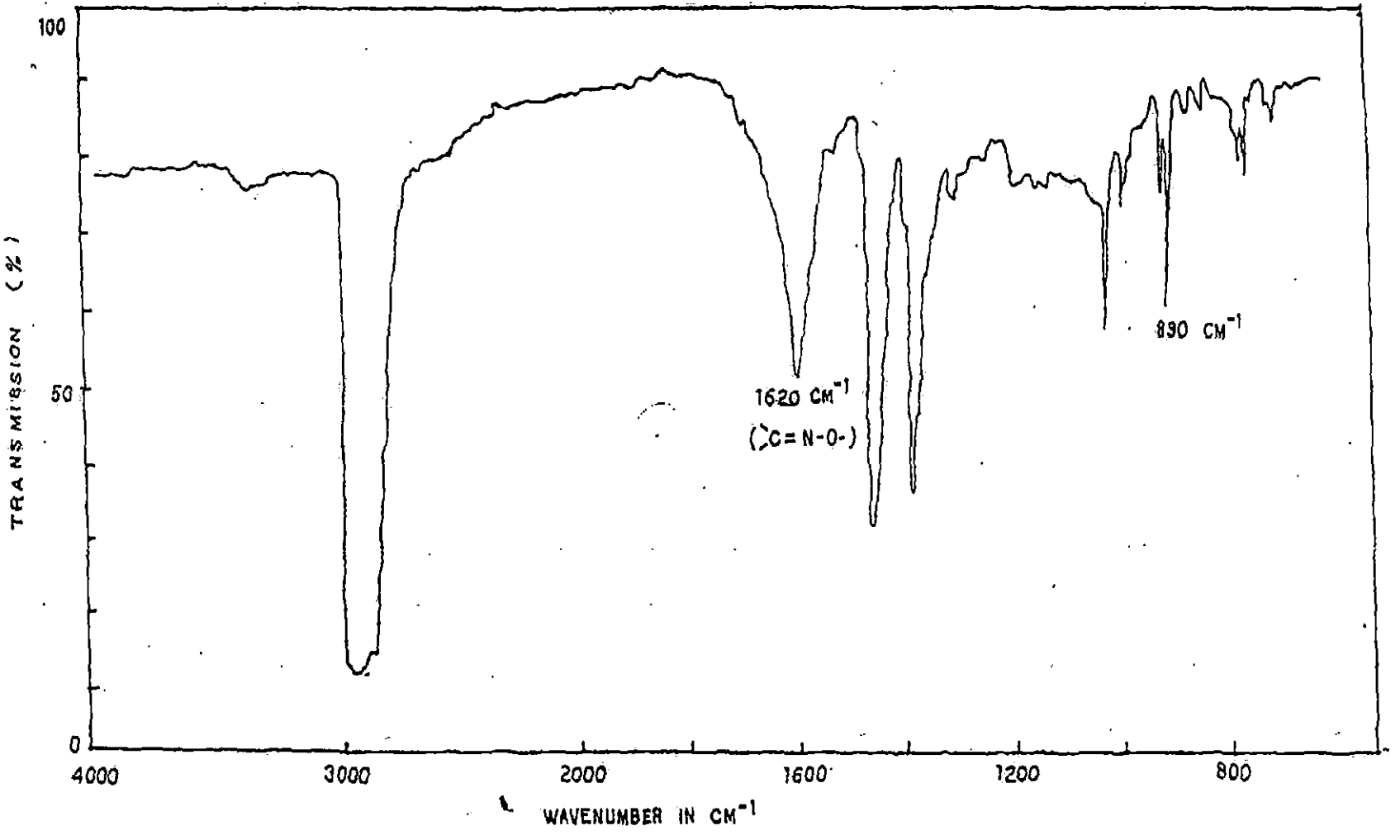


Fig. 15: IR Spectrum of lupane [2,3-C]-1,2,5-oxadiazole (Compound E)

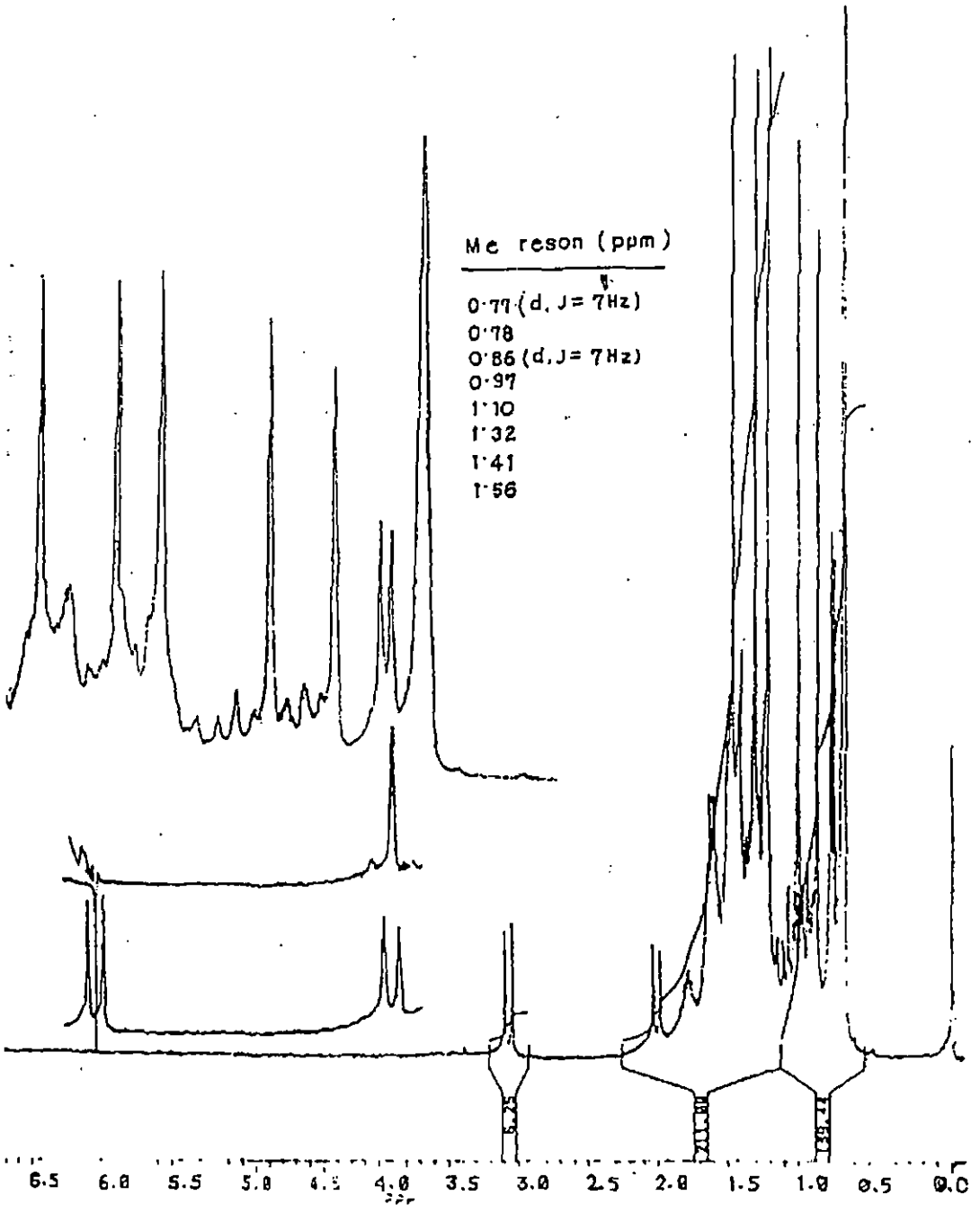


Fig. 16: ^{1}H NMR Spectrum of lupan [2,3-C]-1′,2′,5′-oxadiazole (Compound E)

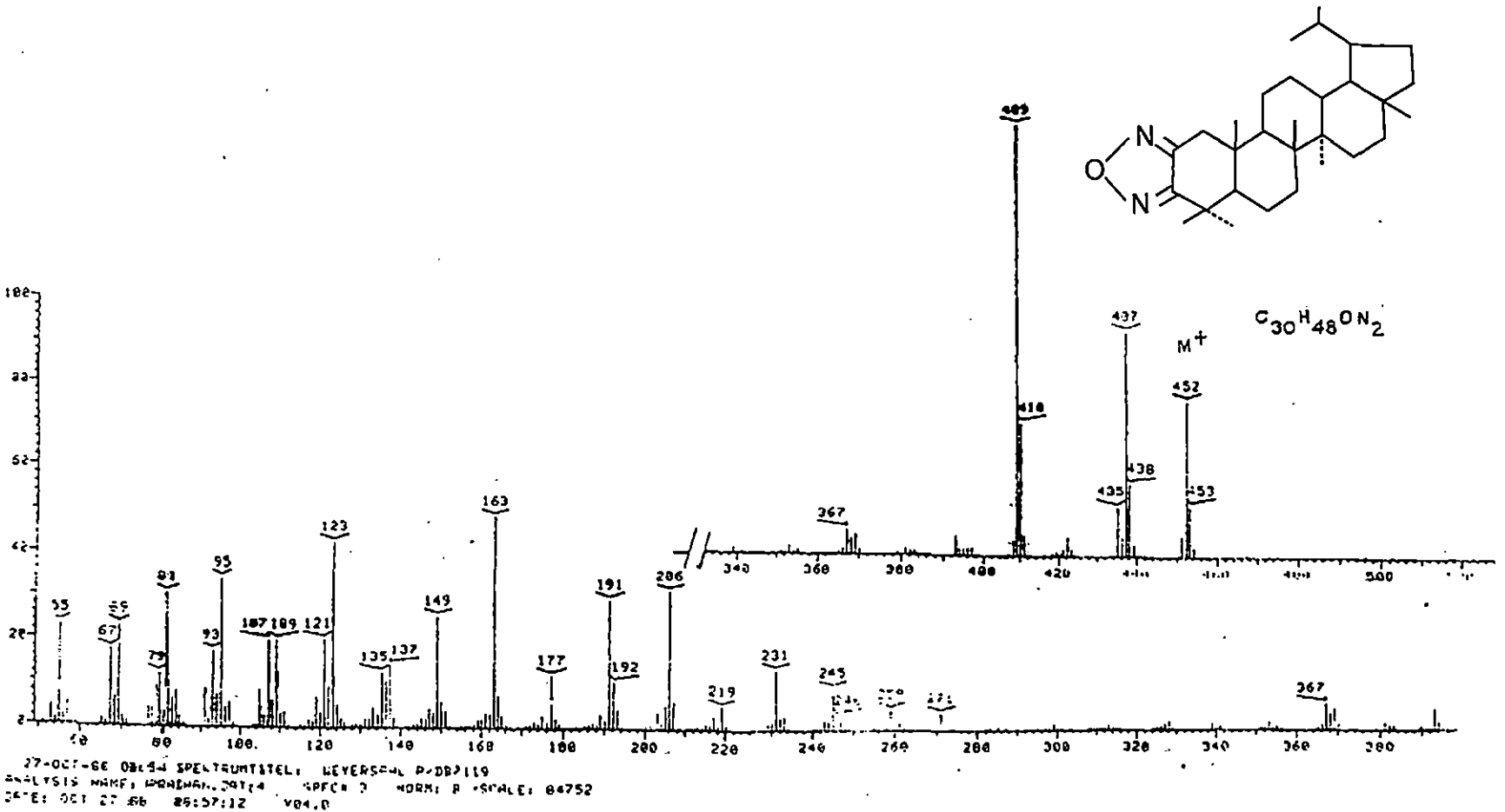
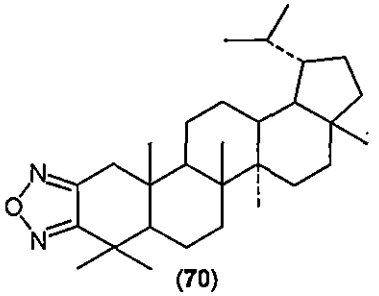


Fig. 17: Mass Spectrum of lupane [2,3-C]-1́,2́,5́-oxadiazole (Compound E)

have been shifted downfield about 0.5 ppm in comparison to that of lupanone 1b. A pair of one proton doublets each at 2.1 (J = 16Hz) ppm and 3.15(J = 16Hz) ppm may be due to the germinal coupling of the C-1 proton which are adjacent to the furazine ring. Thus spectral analysis the structure of the compound has been established as lupan-[2,3-C]-1',2',5'-oxadiazole.



(70)

Lupane-[2,3-C]-1',2',5'-oxadiazole

Section 2: Antimicrobial activity

2.1: Materials and Methods

2.1.1: General Introduction: In the present study three fungal and four bacterial pathogens have been used.The details of the pathogens, their sources and their identification status have been presented in the following tables.

Table 2.5: Pathogenic fungal cultures used

Fungal culture	Source	Identified by	Identification No.
Colletotrichum gloeosporioides	Plant pathology lab (originally isolated from naturally infected brinjal leaf)	Indian Type Culture Collection, IARI New Delhi	ITCC-5446.02
Fusarium equiseti	Plant pathology lab (originally isolated from naturally infected tender brinjal stem)	Indian Type Culture Collection, IARI New Delhi	ITCC-6566.07
Curvularia eragrostidis	Plant pathology lab (originally isolated from naturally infected tender brinjal stem)	Indian Type Culture Collection, IARI New Delhi	ITCC- 4150.2K

Table 2.6: Pathogenic bacterial cultures used

Culture	Source	Identification no./ Identified by	Bacterial culture
RS	American type culture collection	Procured from American type culture collection. Identification no. ATCC-B3123	***Ralstonia solanacearum***
OB5	Plant Pathology Laboratory, Department of Botany, University of North Bengal.	Originally isolated from naturally infected pineapple and identified* by Dr.A.Saha	***Xanthomonas* sp.**
TB3	Plant Pathology Laboratory, Department of Botany, University of North Bengal.	Originally isolated from naturally infected pineapple and identified* by Dr.A.Saha	***Pseudomonas syringae***
PB8	Plant Pathology Laboratory, Department of Botany, University of North Bengal.	Originally isolated from naturally infected pineapple and identified* by Dr.A.Saha	***Erwinia carotovora***

RS, OB5, TB3, PB8 were used as test pathogen throughout the present study.
* Identification was done by biochemical characterization of the three bacteria.

2.1.2 Maintenance of stock cultures:

Colletotrichum gloeosporioides were grown in PDA media at 28±1°C in an incubator for 12 hrs. The growth condition was aerobic. Finally the culture was kept in a refrigerator for storage and short time maintenance. Throughout the present study 12 hrs. old freshly grown cultures were used. Routine subcultures were made at 30 days intervals.

Fusarium equiseti were grown in PDA media at 28±1°C in an incubator for 48 hrs. The growth condition was aerobic. Finally the culture was kept in a refrigerator for storage and short time maintenance. Throughout the present study 12 hrs. old freshly grown cultures were used. Routine subcultures were made at 30 days intervals.

Curvularia eragrostidis were grown in PDA media at 28±1^{0}C in an incubator for 12hrs. The growth condition was aerobic. Finally the culture was kept in a refrigerator for storage and short time maintenance. Throughout the present study 24 hrs. old freshly grown cultures were used. Routine subcultures were made at 45 days intervals.

Ralstonia solanacearum, a G$^-$, nonsporing bacteria were grown in nutrient agar media at 37±1^{0}C in an incubator for 24 hrs. The growth condition was aerobic. Finally the culture was kept in a refrigerator for storage and short time maintenance. Throughout the present study 24 hrs. old freshly grown cultures were used. Routine subcultures were made at 30 days intervals.

Xanthomonas sp, a G$^-$bacteria were grown in nutrient agar media at 37±1^{0}C in an incubator for 24 hrs. The growth condition was aerobic. Finally the culture was kept in a refrigerator for storage and short time maintenance. Throughout the present study 24 hrs. old freshly grown cultures were used. Routine subcultures were made at 30 days intervals.

Pseudomonas syringae, a rod shaped,G$^-$, non spore formaing bacteria were grown in nutrient agar media at 37±1^{0}C in an incubator for 24hrs. The growth condition was aerobic. Finally the culture was kept in a refrigerator for storage and short time maintenance. Throughout the present study 24 hrs. old freshly grown cultures were used. Routine subcultures were made at 30 days intervals.

Erwinia carotovora, a G$^-$, rod shaped bacteria were grown in nutrient agar media at 37±1^{0}C in an incubator for 24hrs. The growth condition was aerobic. Finally the culture was kept in a refrigerator for storage and short time maintenance. Throughout the present study 24 hrs. old freshly grown cultures were used. Routine subcultures were made at 30 days intervals.

2.1.3 Spore germination bioassay:

Ten days old sporulated fungal culture was taken and approximately 3-5ml sterile distilled water was poured in the culture tube aseptically. Gentle scrapping was done by an inoculating needle on the agar surface. After the scrapping, the tube was shaken and

the resultant mixture was strained through cheesecloth. The filtrate was used as spore suspension. The concentration of the spores in the suspension was adjusted by adding sterile distilled water following hemocytometer count.

All the test compounds and their derivatives were subjected to bioassay against three fungal pathogens (*C. gloeosporioides, F. equiseti* and *C. eragrostidis*). The spores of the pathogens were allowed to germinate in sterile distilled water drops mounted on sterile grease free slides kept in a humid chamber in case of control. In experimental sets 30 µl of each test compound (dissolved in DMSO) was placed on the centre of a clean (grease free) microscopic slide. After that, spore suspension was mounted on the slides in the same place where the extract was applied. In solvent control set fresh solvent (DMSO) was placed and subsequently spore suspension was applied. Two small glass rods (60 mm in length) were placed in a 90 mm petridish and the experimental slide was placed on the rods in a uniformly balanced position. Sterile distilled water was carefully poured in the petridish so that the bottom of the slide remained just above the water surface. The petridish was then covered for maintaining humid condition and finally the petridishes were incubated at 28 ± 1^0C. After 48 h of incubation, the slides were stained with lacto phenol-cotton blue and were observed under microscope. Approximately, 200 spores were observed in each slide for germination. The entire experiment was repeated thrice.

2.1.4 Bioassay by Disc diffusion Method:

Two milliliter of spore suspension was poured in a sterile petridish (90 mm diameter) and then 18 ml of the molten PDA medium was poured in the same petridish. The spore suspension and medium was mixed well and was allowed to solidify. After solidification of petridishes filter paper discs (Whatmann 40, 4mm in diameter) were dipped in different concentrations of the test compounds (100, 200, 300, 400 and 500 ppm) and then were placed on the solidified plates. In solvent control sets filter paper discs dipped in pure DMSO was placed. In distilled water control sets filter paper discs dipped in distilled water were placed on the solidified medium surface. Both experimental and control plates were incubated for period as required. Radial growth of each pathogen was measured.

In case of bacterial pathogen nutrient agar was used instead of PDA.

2.1.5. Determination of Minimum inhibitory concentration by agar cup method:

For screening of inhibitory effect of botanicals against test pathogens both spore germination bioassay technique and disc diffusion bioassay technique were followed. Minimun inhibitory concentrations (MIC) of all active components were measured following standard procedures as suggested by Portillo *et al.* 2005.[37]

A sensitive and quick petridish method as suggested by Eloff (1998)[38] was followed to determine the minimum inhibitory concentration of plant extracts against bacteria and fungi. Minimum inhibitory concentration (MIC) was determined by the micro dilution method using serially diluted test compounds. Various concentrations (Viz. 100ppm, 200ppm, 300ppm, 400ppm and 500ppm) of the plant extracts were prepared. Nutrient agar was used for the growth of bacterial strain and potato dextrose agar was used for the growth of fungi. By means of a cork borer (5 mm in diameter) a cup or well was made in a seeded (either by bacterial suspension or by fungal spore suspension) agar plate. The cup or well was filled with 50µl tested compound and was incubated in the incubators meant for bacteria or fungus as applicable. The extract diffused from the cup to a certain extent and inhibited the growth of the pathogen if it contained any antimicrobial properties. It was found that the diameter of the inhibition zone was more when the concentration of the antimicrobial properties was more. The plates containing bacteria were incubated at 37±1°C for 48 hrs. and the plates containing fungi were incubated at 28±1°C for 72hrs. The antimicrobial activity was noted on the basis of the diameter of inhibition zone where no growth was found.

2.2: Results

Compound A, chemical name 'Lupeol', extracted from the bark of *Xanthoxylum budrunga*, belongs to the family Rutaceae [details of extraction procedure have been described in chapter-II, section 1.1]. The bark was used to treat cold, cough, toothache etc. Antifungal and antimicrobial activities of the plant parts were confirmed by pharmacological research. The plant parts also had anti-inflammatory and anti-tumor effect as reported by several scientists [12-17].

From the results presented in table-2.7 it was found that the antifungal activity of lupeol was effective at higher concentrations. Out of the five concentrations tested two concentrations (400 and 500ppm) showed antifungal activity (more than 90% inhibition) in all the three fungal pathogens (*Colletotrichum gloeosporioides, Fusarium equiseti* and *curvularia eragrostidis*). Percent germination of spores gradually decreased with the increasing concentration of the compound. In all the three cases 500 ppm concentration was most effective than the other concentrations.

Table 2.7: Percent inhibition of spore germination of *Colletotrichum gloeosporioides, Fusarium equiseti* and *Curvularia eragrostidis* by lupeol (compound A). (when control raised to 100).

Fungal organism	Concentrations of compound (ppm)	Range of germtube length (micrometer)	percent germination	Percent Inhibition*
Colletotrichum gloeosporioides	100	12-44	85	15±1.06
	200	12-40	32	68±1.50
	300	12-27	21	79±1.20
	400	08-32	05	95±1.90
	500	08-24	04	96±1.04
Fusarium equiseti	100	12-24	84	14±1.72
	200	08-20	18	82±1.38
	300	08-20	17	83±1.92
	400	04-16	10	90±1.45
	500	04-12	05	95±1.40
Curvularia eragrostidis	100	40-88	15	85±1.02
	200	32-80	11	89±1.30
	300	28-68	09	91±1.40
	400	24-56	07	93±1.55
	500	20-48	05	95±1.10

*Data after ± indicate standard error value

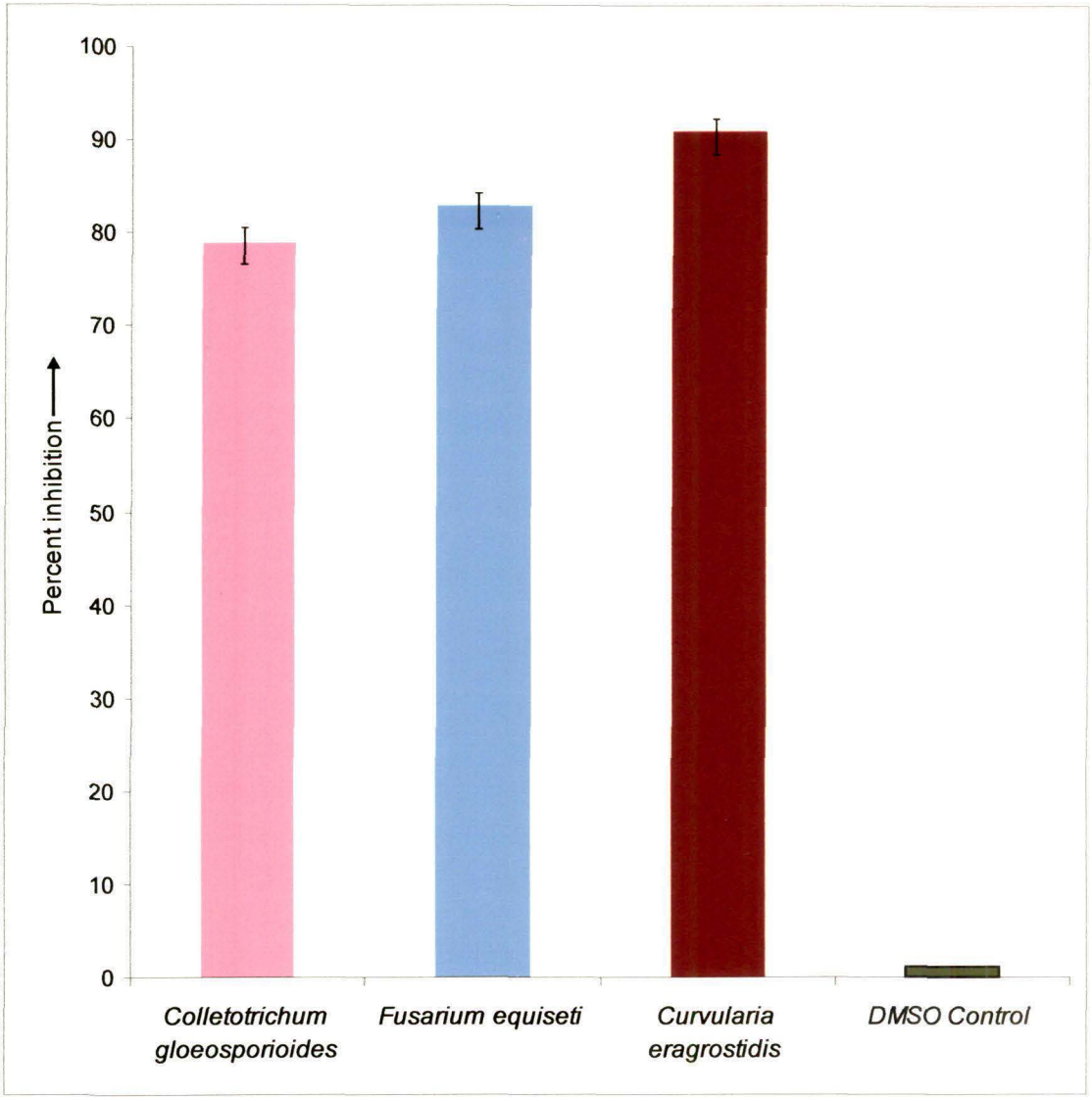


Fig. 18: Percent inhibition of spore germination at 300 ppm concentration of compound A (lupeol).

Table 2.9: Antibacterial activity of Lupeol (compound-A)

Bacterial organism	Concentrations of compound (ppm)	Diameter of Inhibition zone (cm)*
Ralstonia solanacearum	Control	-
	100	0.4
	200	0.6
	300	0.9
	400	1.2
	500	1.4
Xanthomonas sp	control	-
	100	1.5
	200	1.7
	300	1.9
	400	2.1
	500	2.2
Pseudomonas syringae	Control	-
	100	1.0
	200	1.2
	300	1.5
	400	1.6
	500	1.7
Erwinia carotovora	Control	-
	100	1.1
	200	1.4
	300	1.7
	400	1.9
	500	2.2

*mean of three replications; - indicates no inhibition zone formed

Compound B, first derivative of mother compound lupeol identical as 2,2-dibromo lupanone obtained from the following reactions: by hydrogenation of lupeol, lupanol was prepared. From lupanol, lupanone was formed by Jone's oxidation. Treating lupanone with N-bromosuccinimide, in dimethylsulfoxide-chloroform mixture, compound-B(2,2–dibromo lupanone) was prepared.

Results of spore germination bioassay have been presented in table-2.10. From the results it was found that the antifungal activity of 2,2-dibromolupanone (compound-B) was effective at all the five concentrations. But three concentrations (300, 400 and 500ppm) showed significant antifungal activity (89-99% inhibition of spore germination) against the three fungal pathogens (*Colletotrichum gloeosporioides, Fusarium equiseti* and *curvularia eragrostidis*). Percent germination of spores gradually decreased with the

increasing concentration of the compound. In all the three cases 500 ppm concentration was more effective than the other concentrations.

Table2.10: Percent inhibition of spore germination of *Colletotrichum gloeosporioides, Fusarium equiseti* and *Curvularia eragrostidis* by 2,2 –dibromo lupanone (compound B) (when control raised to 100).

Fungal organism	Concentrations of compound (ppm)	Range of germtube length (micrometer)	percent germination	Percent Inhibition*
Colletotrichum gloeosporioides	100	12-48	23	77±1.10
	200	08-44	05	95±1.34
	300	08-40	03	97±1.58
	400	04-36	03	97±1.67
	500	04-28	01	99±1.87
Fusarium equiseti	100	12-20	27	73±1.60
	200	08-32	12	88±1.77
	300	08-32	11	89±1.98
	400	04-12	09	91±1.45
	500	04-12	06	94±1.65
Curvularia eragrostidis	100	36-100	13	87±1.20
	200	28-72	08	92±1.40
	300	24-68	07	93±1.44
	400	20-60	06	94±1.02
	500	16-44	04	96±1.19

*Data after ± indicate standard error value

From the results presented in the table 2.11 and plate-2 it was found that the antifungal activity of 2, 2 dibromolupanone (compound B) was very much significant. Out of five concentrations tested three concentrations (300,400,500ppm) of the compound B were very effective and showed antifungal activity (inhibition zones diameter of 1.0-2.0cm) against all the three tested fungal pathogens (*Colletotrichum gloeosporioides, Fusarium equiseti and Curvularia eragrostidis*). Diameter of inhibition

zones were increased with the increasing concentration of the compound. Best antifungal activity was obtained against all the three fungus when 500 ppm concentration was used.

In table 2.12 and plate-2 the results of antibacterial activity of 2,2-dibromolupanone (compound-B) against four bacteria (*R. solanacearum, Xanthomonas sp, P. syringae* and *E. carotovora*) have been presented. Out of five concentrations tested, three concentrations (300,400,500ppm) of the compound were proved to be significant (diameter of inhibition zones ranged from 1.2-2.3cm). The diameter of inhibition zones were gradually increased with increasing concentrations of the compound-B. Highest antibacterial activity was observed at 500ppm concentration of compound-B.

Table 2.11: Antifungal activity of 2,2 –dibromo lupanone (compound B)

Fungal organism	Concentrations of compound (ppm)	Diameter of inhibition zone(cm)*
Colletotrichum gloeosporioides	100	0.6
	200	0.8
	300	1.0
	400	1.2
	500	1.3
Fusarium equiseti	100	1.3
	200	1.5
	300	1.6
	400	1.7
	500	1.9
Curvularia eragrostidis	100	1.2
	200	1.5
	300	1.7
	400	1.8
	500	2.0

*mean of three replications

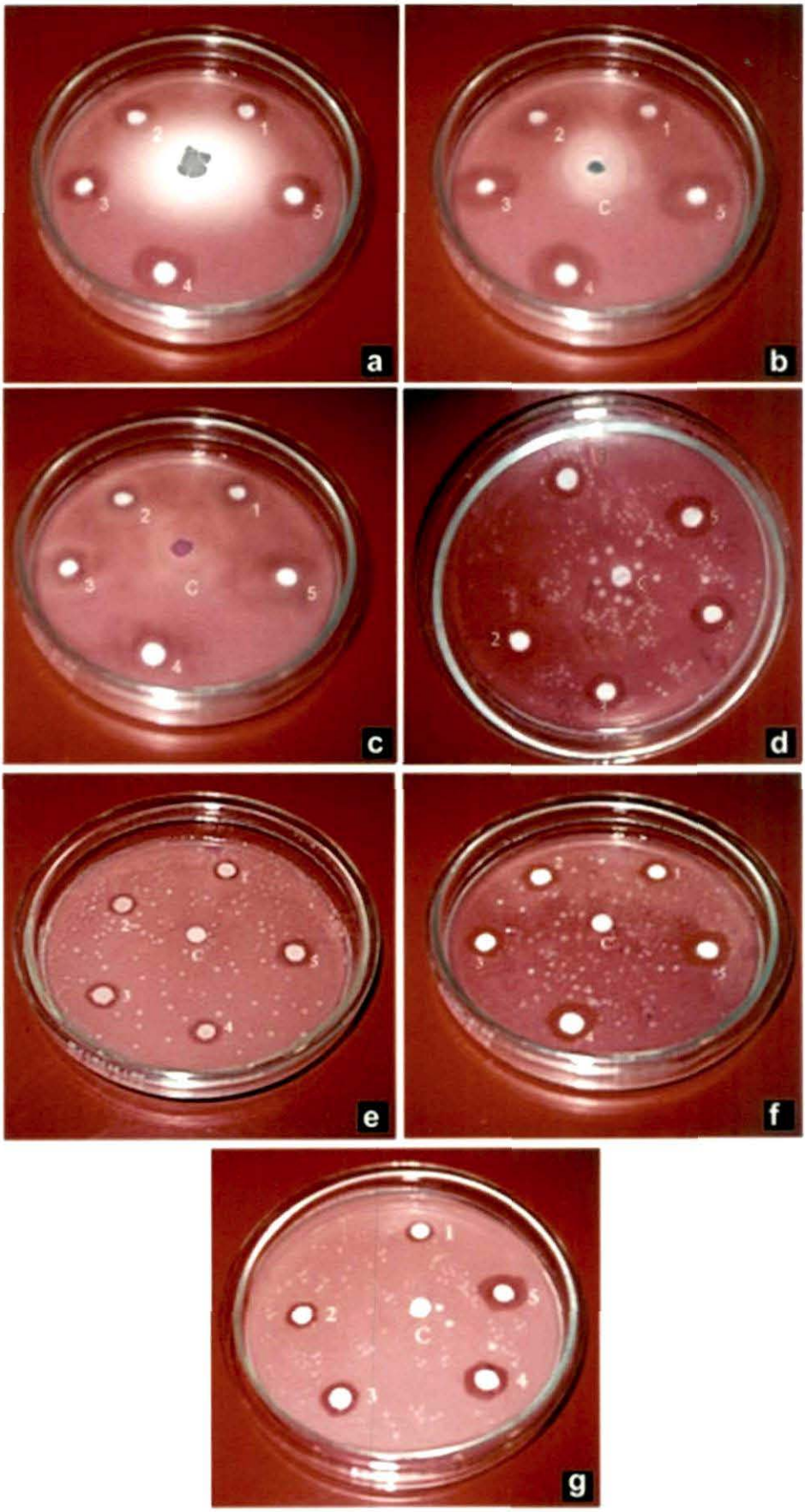


Plate 1: Disc diffusion test for anti microbial activity of compound A: (Lupeol) at five different concentrations* against (a) *Colletotrichum gloeosporioides* (b) *Curvularia eragrostidis* (c) *Fusarium equiseti* (d) *Ralstonia solanacearum* (e) *Pseudomonas syringae* (f) *Erwinia carotovora*. (g) *Xanthomonas* sp.

* 1=100ppm, 2=200 ppm, 3=300 ppm, 4=400 ppm, 5=500ppm, c=DMSO control.

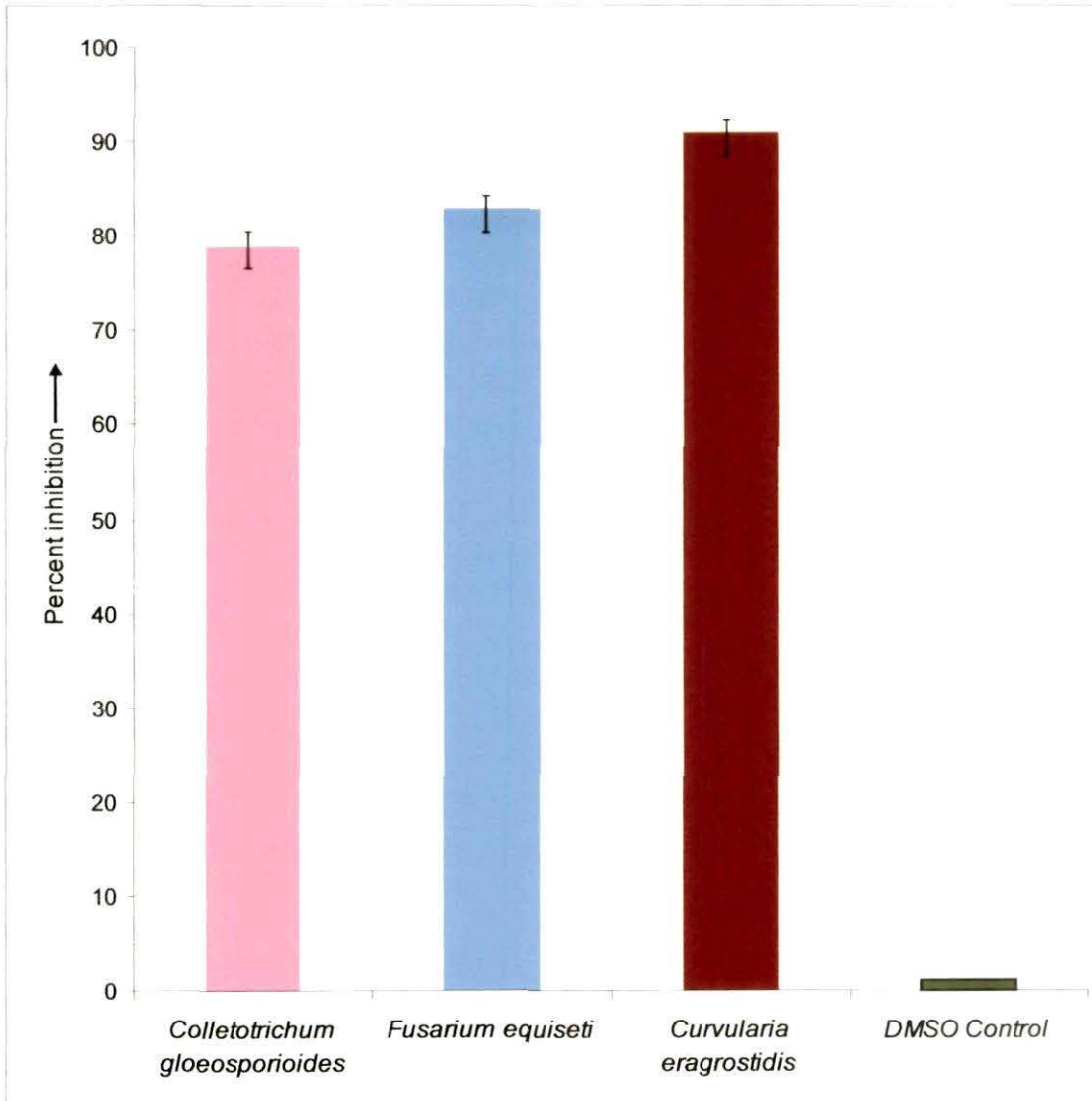


Fig. 18: Percent inhibition of spore germination at 300 ppm concentration of compound A (lupeol).

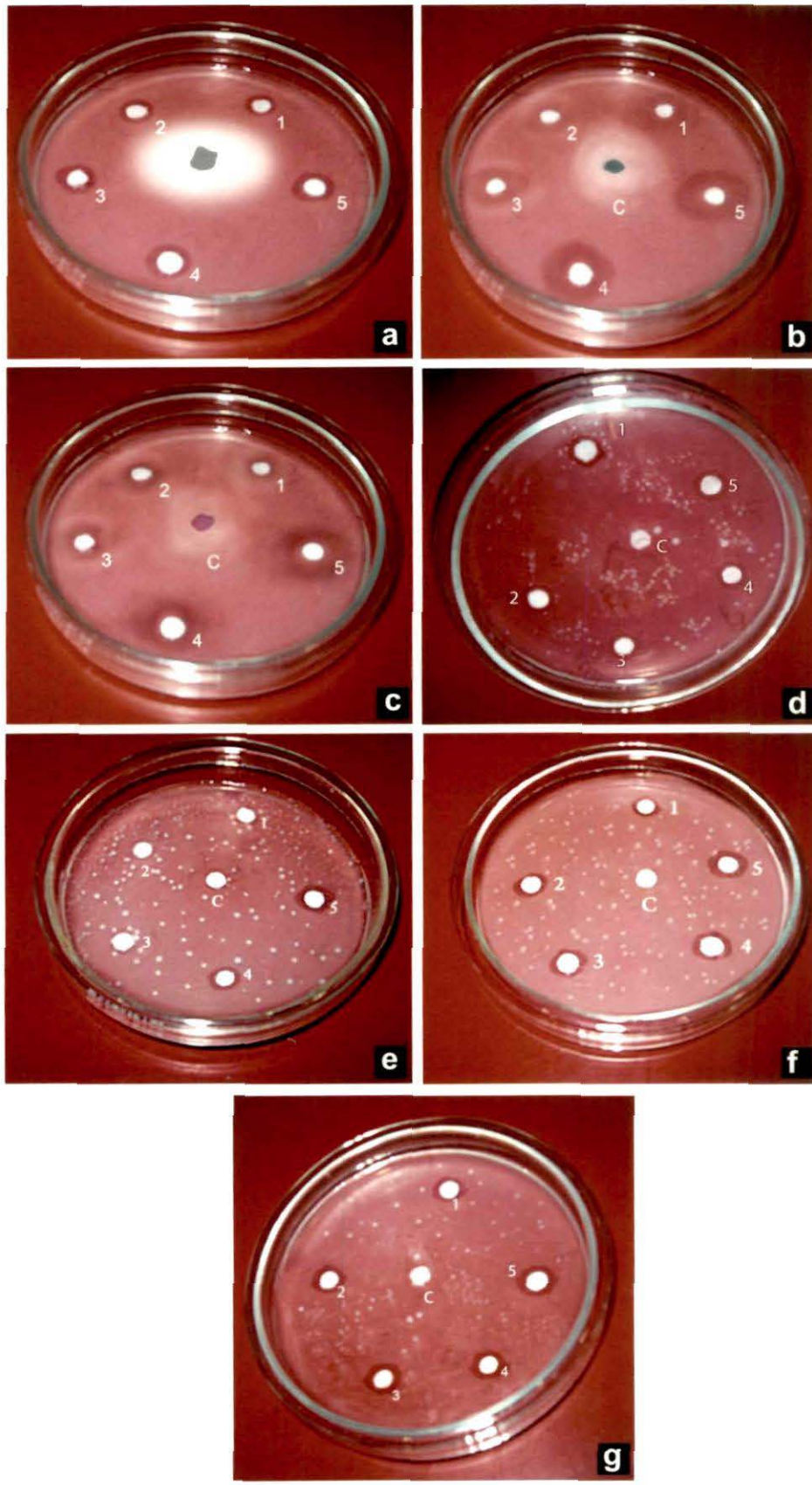


Plate 2: Disc diffusion test for anti microbial activity of compound B: (2,2-dibromo lupanone); at five different concentrations* against (a) *Colletotrichum gloeosporioides* (b) *Curvularia eragrostidis* (c) *Fusarium equiseti* (d) *Ralstonia solanacearum* (e) *Pseudomonas syringae* (f) *Erwinia carotovora*. (g) *Xanthomonas* sp.

* 1=100ppm, 2=200 ppm, 3=300 ppm, 4=400 ppm, 5=500ppm, c=DMSO control.

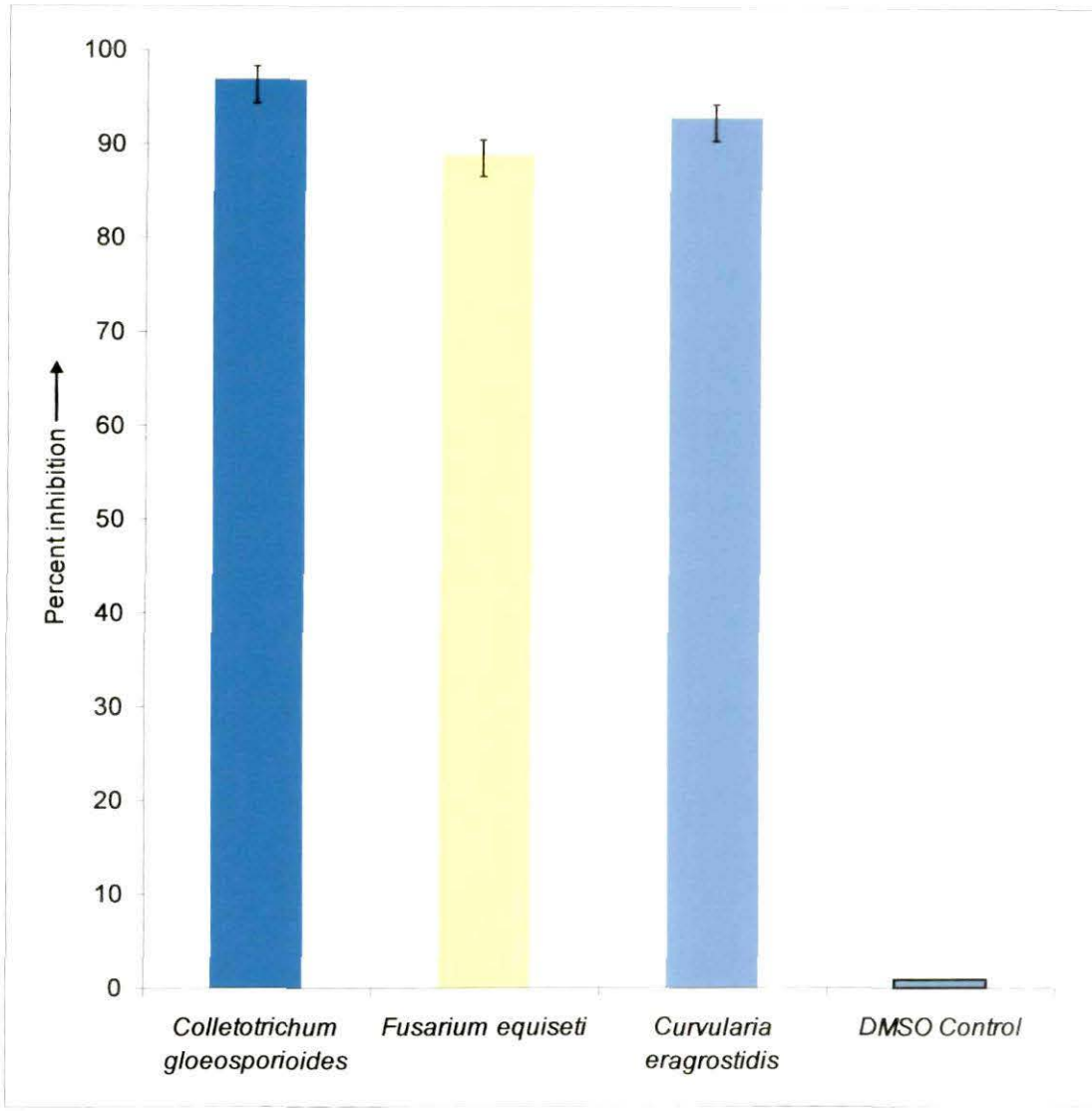


Fig. 19: Percent inhibition of spore germination at 300 ppm concentration of compound B (2,2-dibromolupanone).

Table 2.12: Antibacterial activity of 2,2–dibromo lupanone (compound B)

Bacterial organism	Concentrations of compound (ppm)	Diameter of inhibition zone (cm)*
Ralstonia solanacearum	Control	-
	100	1.0
	200	1.2
	300	1.5
	400	1.8
	500	2.0
Xanthomonas sp	control	-
	100	1.2
	200	1.4
	300	1.7
	400	1.9
	500	2.2
Pseudomonas syringae	Control	-
	100	0.8
	200	1.0
	300	1.2
	400	1.5
	500	1.7
Erwinia carotovora	Control	-
	100	1.2
	200	1.5
	300	1.8
	400	2.0
	500	2.3

*mean of three replications; - indicates no inhibition zone formed

Compound-C, the second derivative of lupeol was identified as 2α-bromolupanone. 2αbromolupanone was obtained from N-bromosuccinimide by treatment of lupanone in DMSO-$CHCl_3$ as mentioned in the chemical derivative preparation section. From the results, presented in the table 2.13 it was evident that the antifungal activity of 2α–bromolupanone (compound C) was effective in controlling plant

pathogens. In all the five concentrations tested compound-C showed antifungal activity against the three fungal pathogens (*Colletotrichum gloeosporioides, Fusarium equiseti and Curvularia eragrostidis*). Inhibition of spore germination was gradually increased with the increasing concentrations of the compound. Although all concentrations of the compound were effective but 500 ppm concentration showed more than 95% inhibition of spore germination.

Table 2.13: Percent inhibition of spore germination of *Colletotrichum gloeosporioides, Fusarium equiseti* and *Curvularia eragrostidis* by 2α-bromolupanone (compound C) (when control raised to 100).

Fungal organism	Concentrations of compound (ppm)	Range of germtube length (micrometer)	percent germination	Percent Inhibition*
Colletotrichum gloeosporioides	100	12-48	21	79±1.12
	200	12-48	17	83±1.79
	300	08-40	07	93±1.60
	400	04-36	06	94±1.77
	500	04-28	02	98±1.88
Fusarium equiseti	100	12-20	17	83±1.98
	200	08-16	12	88±1.75
	300	08-16	10	90±1.56
	400	04-12	06	94±1.36
	500	04-08	02	98±1.30
Curvularia eragrostidis	100	36-84	20	80±1.57
	200	32-72	12	88±1.10
	300	28-64	11	89±1.35
	400	24-56	10	90±1.90
	500	20-44	05	95±2.00

*Data after ± indicate standard error value

Antifungal activity of 2α-bromolupanone (compound-C) was also tested by disc diffusion method on agar plates. Results presented in the table 2.14 and plate-3 showed that compound-C was antifungal against *C. gloeosporioides* and *F. equiseti* but it was not effective against *C.eragrostidis.* Compound-C was tested for antifungal activity at five

different concentrations (100,200,300,400,500ppm). Compound-C at concentration below 300ppm did not show any antifungal activity. Diameter of inhibition zones ranged from 0.8-2.1 cm when concentrations of 300,400 and 500ppm of the compound-C were tested against the two pathogens mentioned above.

The results of antibacterial activity of 2α–bromolupanone (compound-C) presented in table 2.15 and plate-3 indicated that Compound-C was effective against three bacteria (*R. solanacearum, Xanthomonas sp, E.carotovora*). Out of five tested concentrations, two concentrations (400 and 500ppm) of the compound were proved to be significant against all three bacteria.It was evident from the results that compound-C was very less effective against *P. syringae.* Only 500ppm concentration of the compound-C showed its activity against *P. syringae.*

Table 2.14: Antifungal activity of 2α-bromolupanone (Compound-C)

Fungal organism	Concentrations of Compound (ppm)	Diameter of inhibition zone (cm)*
Colletotrichum gloeosporioides	100	-
	200	-
	300	0.8
	400	1.2
	500	1.4
Fusariumequiseti	100	1.5
	200	1.5
	300	1.6
	400	2.0
	500	2.1
Curvularia eragrostidis	100	-
	200	-
	300	-
	400	-
	500	-

*mean of three replications; - indicates no inhibition zone formed

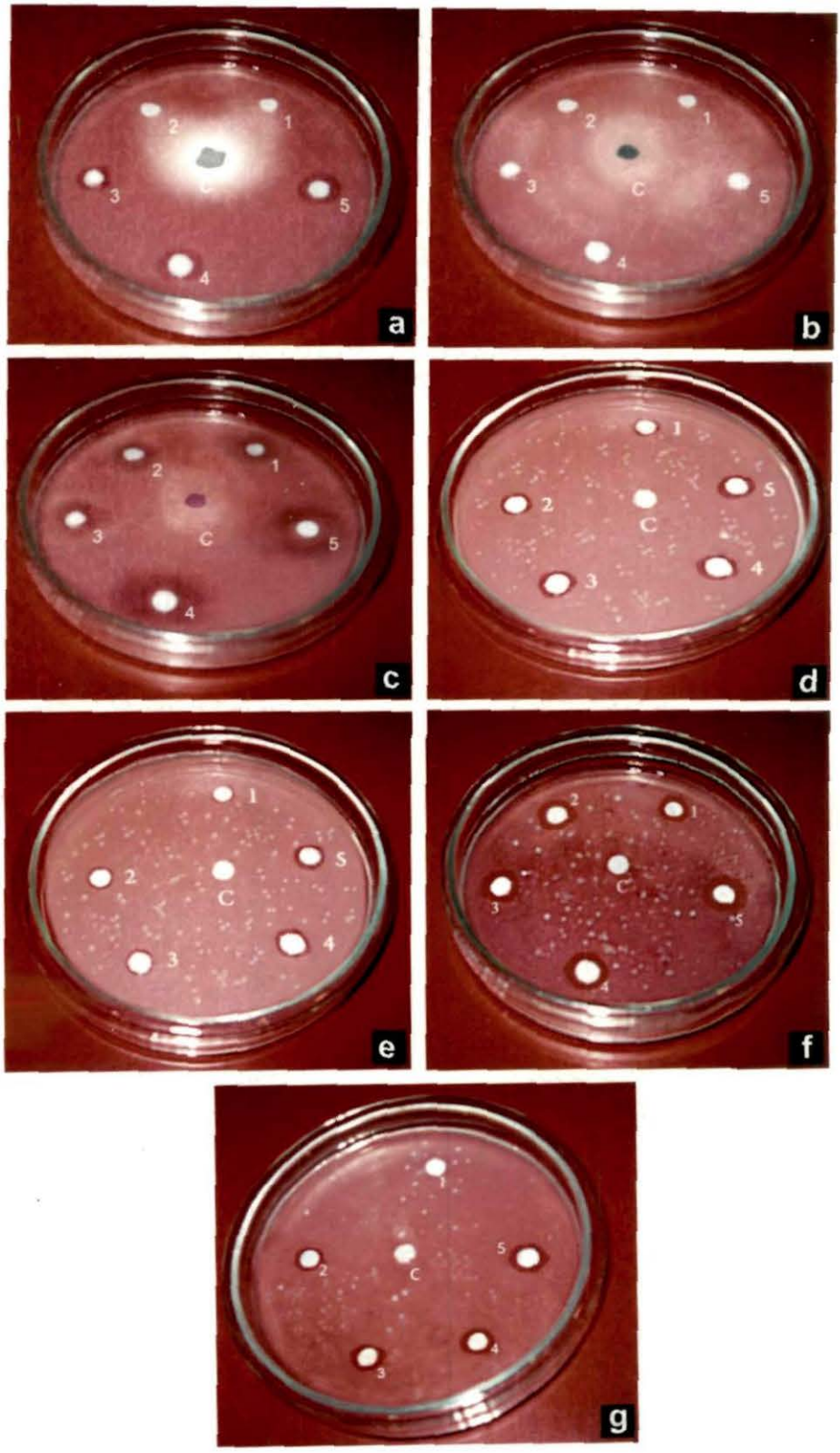


Plate 3: Disc diffusion test for anti microbial activity of compound C: (2α bromo lupanone); at five different concentrations* against (a) *Colletotrichum gloeosporioides* (b) *Curvularia eragrostidis* (c) *Fusarium equiseti* (d) *Ralstonia solanacearum* (e) *Pseudomonas syringae* (f) *Erwinia carotovora*. (g) *Xanthomonas* sp.

* 1=100ppm, 2=200 ppm, 3=300 ppm, 4=400 ppm, 5=500ppm, c=DMSO control.

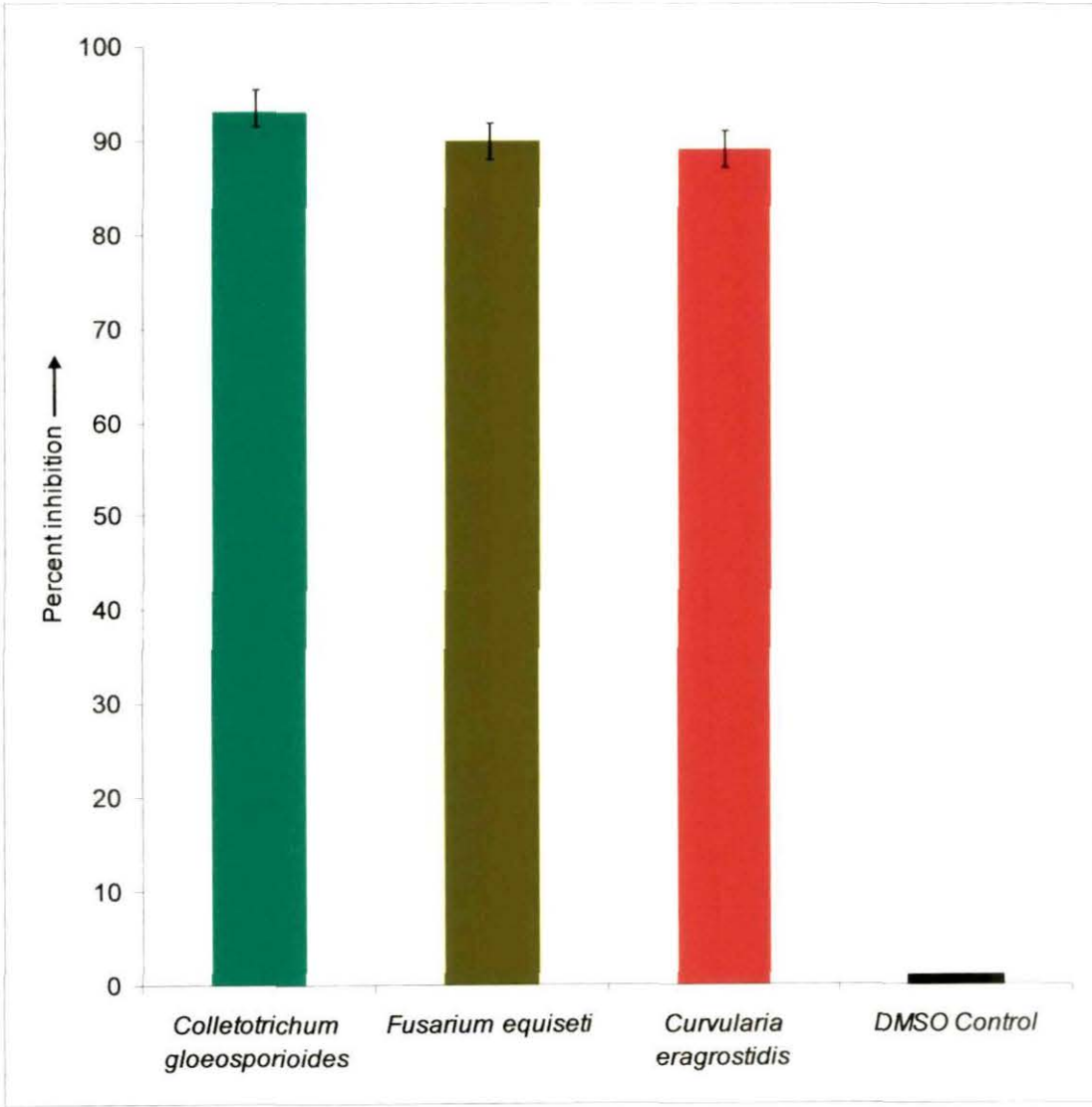


Fig. 20: Percent inhibition of spore germination at 300 ppm concentration of compound C (2α-bromolupanone).

Table 2.15: Antibacterial activity of 2α-bromolupanone (Compound "C")

Bacterial organism	Concentrations of compound (ppm)	Diameter ofinhibition zone (cm) *
Ralstonia solanacearum	Control	-
	100	0.4
	200	0.4
	300	0.8
	400	1.0
	500	1.3
Xanthomonas sp	control	-
	100	0.4
	200	0.8
	300	1.0
	400	1.2
	500	1.3
Pseudomonas syringae	Control	-
	100	0.4
	200	0.4
	300	0.4
	400	0.9
	500	1.1
Erwinia carotovora	Control	-
	100	0.7
	200	0.9
	300	1.2
	400	1.3
	500	1.5

*mean of three replications; - indicates no inhibition zone formed

Compound-D, the third derivative of mother compound lupeol, (identical as lup-1(2)-en-3-one) obtained from the reaction: Dehydro bromination of 2α-bromo lupanone with lithium bromide-N,N-dimethyl formamide.

From the results presented in the table 2.16 it was found that the antifungal activity of lup-1(2)-en-3-one (Compound-D) was positive. Out of five concentrations

tested three concentrations (300,400,500ppm) of the compound D were very much effective and showed antifungal activity (89-97% inhibition of spore germination) against all the three fungal pathogens (*Colletotrichum gloeospoerioides,Fusarium equiseti and Curvularia eragrostidis*) tested. Percent germination was reduced gradually with increasing concentration of the compound. Although 300,400 and 500ppm concentrations of the compound were effective but 500 ppm concentrations was most effective.

Table 2.16: Percent inhibition of spore germination of *Colletotrichum gloeosporioides, Fusarium equiseti* and *Curvularia eragrostidis* by Lup-1(2)-en-3-one (Compound"D") (when control raised to 100)

Fungal organism	Concentrations of compound (ppm)	Range of germtube length (micrometer)	percent germination	Percent Inhibition*
Colletotrichum gloeosporioides	100	12-44	26	74±1.97
	200	08-40	12	88±2.01
	300	08-36	11	89±1.85
	400	04-32	08	92±1.66
	500	04-28	04	96±1.44
Fusarium equiseti	100	08-24	25	75±2.00
	200	08-20	15	85±1.73
	300	08-16	10	90±1.32
	400	04-16	03	97±1.56
	500	04-12	02	98±1.40
Curvularia eragrostidis	100	40-96	13	87±1.75
	200	32-80	11	89±1.30
	300	28-52	09	91±1.99
	400	24-48	06	94±1.45
	500	20-32	05	95±1.77

*Data after ± indicate standard error value

From the results presented in the table 2.17 it was found that the antifungal activity of **Lup-1(2)-en-3-one** (Compound-D) was very much effective against the pathogens tested. Diameter of inhibition zones ranged between 0.9-2.5cm against all the three tested fungal pathogens (*Colletotrichum gloeosporioides, Fusarium equiseti* and *Curvularia eragrostidis*). Diameter of inhibition zones was increased with the increasing concentration of the compound.

Results of antibacterial activity of Lup-1(2)-en-3-one (Compound-D) through disc diffusion method have been presented in table 2.18. Compound- D showed significant activity in connection of 300ppm concentration and above. Diameter of inhibition zones of the effective concentrations ranged from 0.9-1.5cm against the four test pathogens (*R. solanacearum, Xanthomonas sp, Pseudomonas syringae, E. carotovora*).

Table 2.17: Antifungal activity by Lup-1(2)-en-3-one (Compound"D")

Fungal organism	Concentrations of Compound (ppm)	Diameter of inhibition zone (cm)*
Colletotrichum gloeosporioides	100	1.4
	200	1.6
	300	1.7
	400	1.9
	500	2.1
Fusarium equiseti	100	1.9
	200	2.0
	300	2.2
	400	2.3
	500	2.5
Curvularia eragrostidis	100	0.9
	200	0.9
	300	1.1
	400	1.2
	500	1.4

*mean of three replications

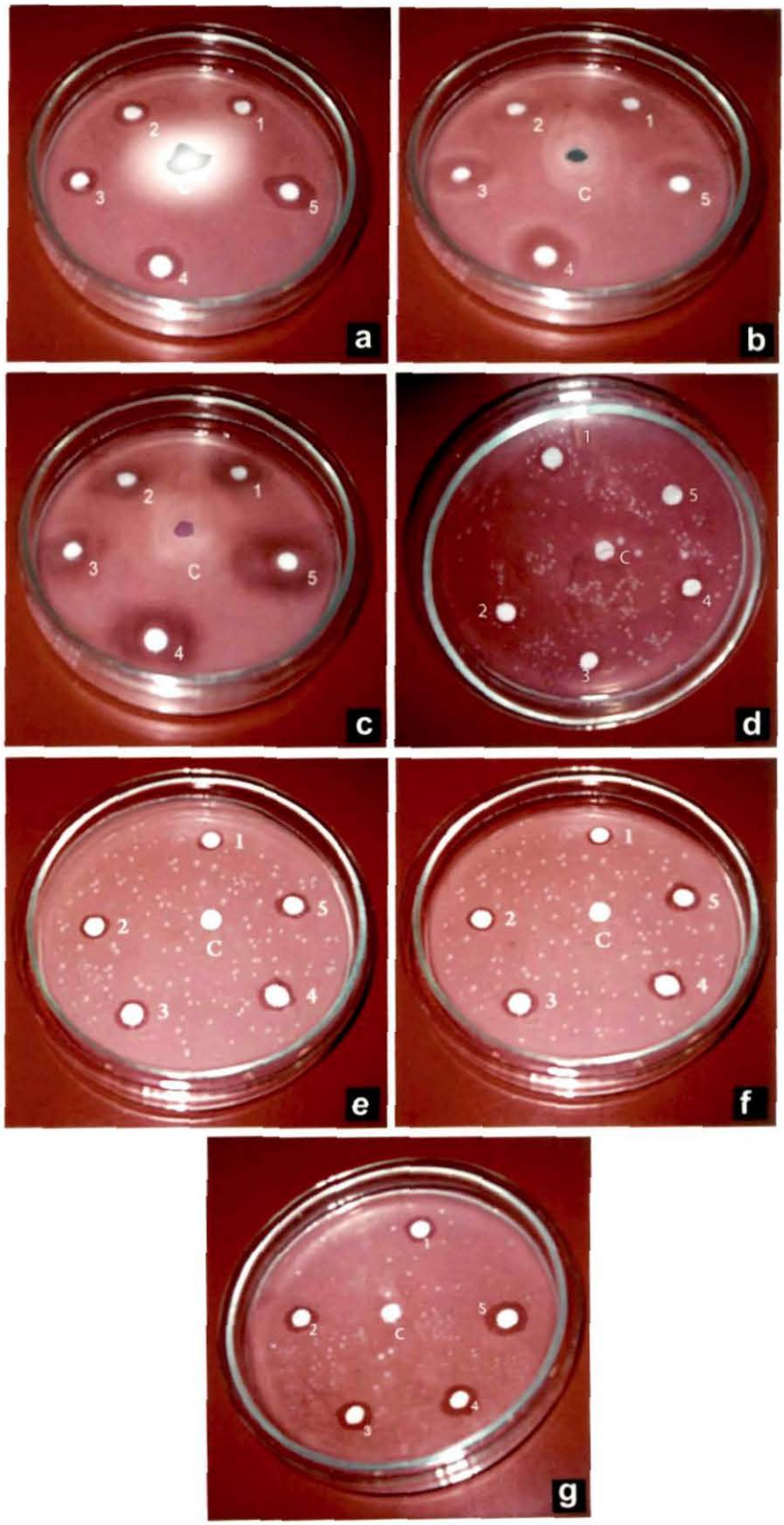


Plate 4: Disc diffusion test for anti microbial activity of compound D: (lup-1-2-en-3-one); at five different concentrations* against (a) *Colletotrichum gloeosporioides* (b) *Curvularia eragrostidis* (c) *Fusarium equiseti* (d) *Ralstonia solanacearum* (e) *Pseudomonas syringae* (f) *Erwinia carotovora*. (g) *Xanthomonas* sp.

* 1=100ppm, 2=200 ppm, 3=300 ppm, 4=400 ppm, 5=500ppm, c=DMSO control.

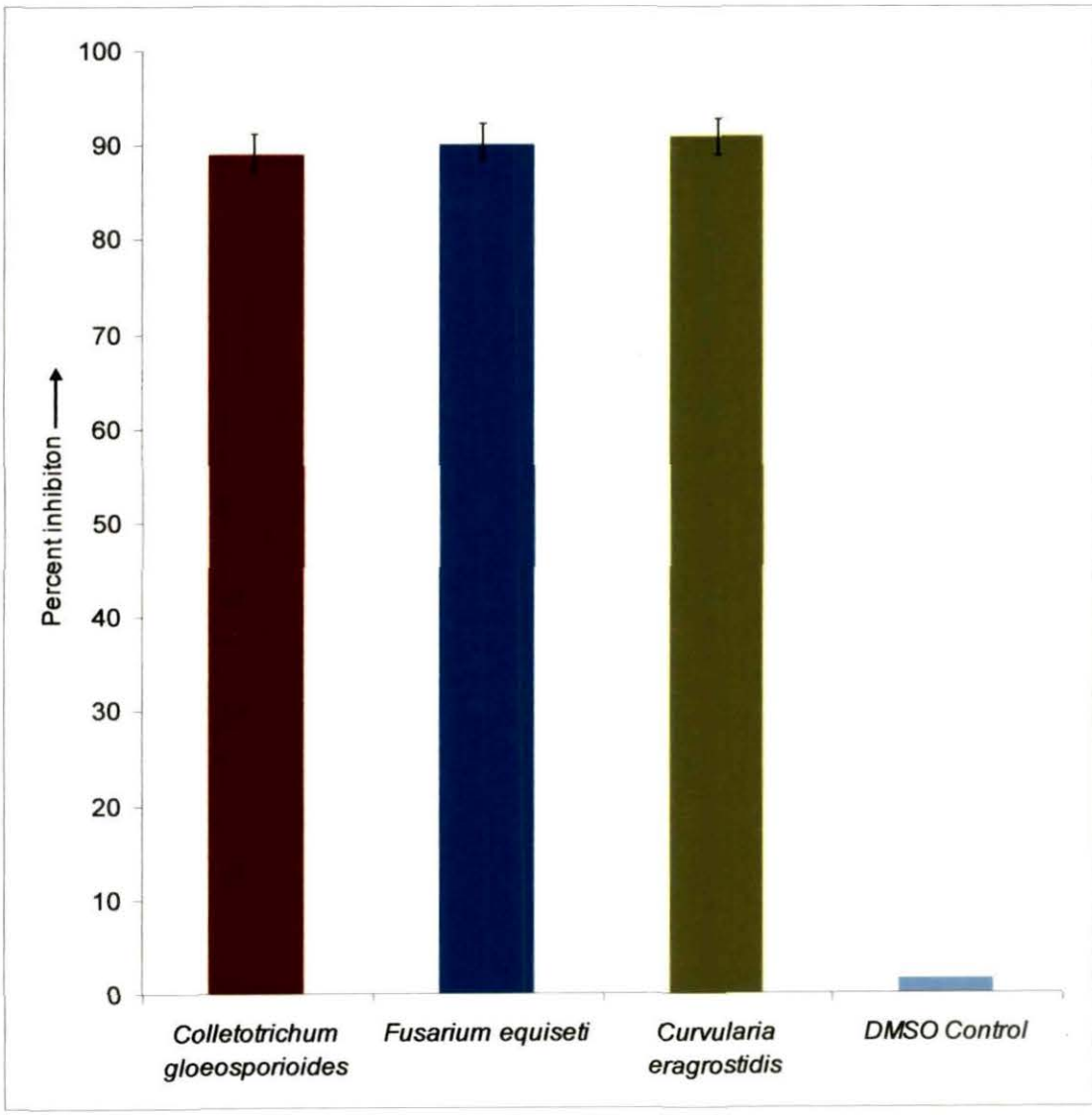


Fig. 21: Percent inhibition of spore germination at 300 ppm concentration of compound D (Lup-1-(2)-en-3-one).

Table 2.18: Antibacterial activity of Lup-1(2)-en-3-one (Compound"D")

Bacterial organism	Concentrations of compound (ppm)	Diameter of inhibition zone (cm)*
Ralstonia solanacearum	Control	-
	100	0.4
	200	0.6
	300	0.9
	400	1.1
	500	1.3
Xanthomonas sp	control	-
	100	0.6
	200	0.8
	300	1.1
	400	1.2
	500	1.5
Pseudomonas syringae	Control	-
	100	0.4
	200	0.7
	300	0.9
	400	0.9
	500	1.2
Erwinia carotovora	Control	-
	100	0.7
	200	0.8
	300	1.0
	400	1.2
	500	1.4

*mean of three replications; - indicates no inhibition zone formed

Lupan-[2,3-C]-1',2',5'-oxadiazole (compound-E) was prepared following the chemical methods as described earlier. Compound-E was subjected to bioassays. The results of the spore germination bioassay of compound-E have been presented in Table

2.19. All the five concentrations tested were effective in controlling spore germination of the three fungal pathogens (*Colletotrichum gloeosporioides, Fusarium equiseti* and *Curvularia eragrostidis*). Lowest concentration (100ppm) of the compound-E reduced 87%, 88% and 90% of spore germination in case of *C. gloeosporioides, F. equiseti* and *C. eragrostidis* respectively.

Table 2.19: Percent inhibition of spore germination of *Colletotrichum gloeosporioids, Fusarium equiseti* and *Curvularia eragrostidis* by lupane-[2,3-C]-1′,2′,5′-oxadiazole (Compound-E) (when control raised to 100)

Fungal organism	Concentrations of compound (ppm)	Range of germtube length (micrometer)	percent germination	Percent Inhibition*
Colletotrichum gloeosporioides	100	08-64	10	90±1.54
	200	08-56	05	95 ±1.90
	300	08-40	04	96 ±1.22
	400	08-36	01	99 ±1.47
	500	08-24	-	100 ±1.00
Fusarium equiseti	100	08-20	13	87 ±1.82
	200	08-20	09	91 ±1.59
	300	04-16	06	94 ±1.32
	400	04-12	03	97 ±1.77
	500	04-12	01	99 ±1.50
Curvularia eragrostidis	100	40-120	12	88 ±1.60
	200	36-88	07	93 ±1.66
	300	28-64	03	97 ±1.80
	400	16-40	02	98 ±1.99
	500	16-32	00	100 ±1.00

*Data after ± indicate standard error value; - indicates no fungal spore formed

Antifungal and antibacterial activity of lupane-[2,3-C]-1′,2′,5′-oxadiazole (compound E) were also tested against growth of three different fungal pathogens (*Colletotrichum gloeosporioides, Fusarium equiseti and Curvularia eragrostidis*) and four bacterial pathogens (*R.solanacearum,Xanthomonas sp.,P.syringae* and

E.carotovora). The results of the experiments have been presented in table 2.20, table-2.21 and plate-5. From the results, it was found that the antifungal activity of Compound-E was also very effective. All the five concentrations of the compound-E could significantly check the growth of *C. gleosporioides* and *F. equiseti.* But growth of *C. eragrostidis* was cheked by the compound only at 300ppm concentration and above.

In case of antibacterial test (table 2.21) of the compound it was found that growth of *Pseudomonas* and *Xanthomonas* was better controlled than the other two bacteria tested (*R.solanacearum* and *E.carotovora*). However, at highest concentration (500ppm) of the compound growth of all the four bacteria were controlled.

Table 2.20: Antifungal activity of lupane[2,3-C]-1',2',5'--oxadiazole (compound E)

Fungal organism	Concentrations of compound (ppm)	Diameter of inhibition zone* (cm)
Colletotrichum gleosporioides	100	1.6
	200	1.8
	300	2.0
	400	2.1
	500	2.3
Fusarium equiseti	100	1.4
	200	1.5
	300	1.6
	400	1.9
	500	2.0
Curvularia eragrostidis	100	-
	200	-
	300	0.6
	400	0.8
	500	1.0

*mean of three replications; - indicates no inhibition zone formed

Table 2.21: Antibacterial activity of lupane[2,3-C]-1′,2′,5′-oxadiazole(compound E)

Bacterial organism	Concentrations of compound (ppm)	Diameter of inhibition zone* (cm)
Ralstonia solanacearum	Control	-
	100	0.6
	200	0.9
	300	1.0
	400	1.2
	500	1.5
Xanthomonas sp.	control	-
	100	1.3
	200	1.5
	300	1.8
	400	2.0
	500	2.2
Pseudomonas syringae	Control	-
	100	1.0
	200	1.3
	300	1.6
	400	1.8
	500	2.0
Erwinia carotovora	Control	-
	100	0.6
	200	0.8
	300	1.2
	400	1.5
	500	2.0

*mean of three replications; - indicates no inhibition zone formed

Phytotoxicity test of Lupeol and its derivatives:

Phytotoxicity of the five compounds was tested in tomato plants (of Priya variety popularly cultivated in the present study area). To test phytotoxicity, tomato plants were grown in earthen pots [size 15cm (diameter) and 15 cm (height)]. The pots were

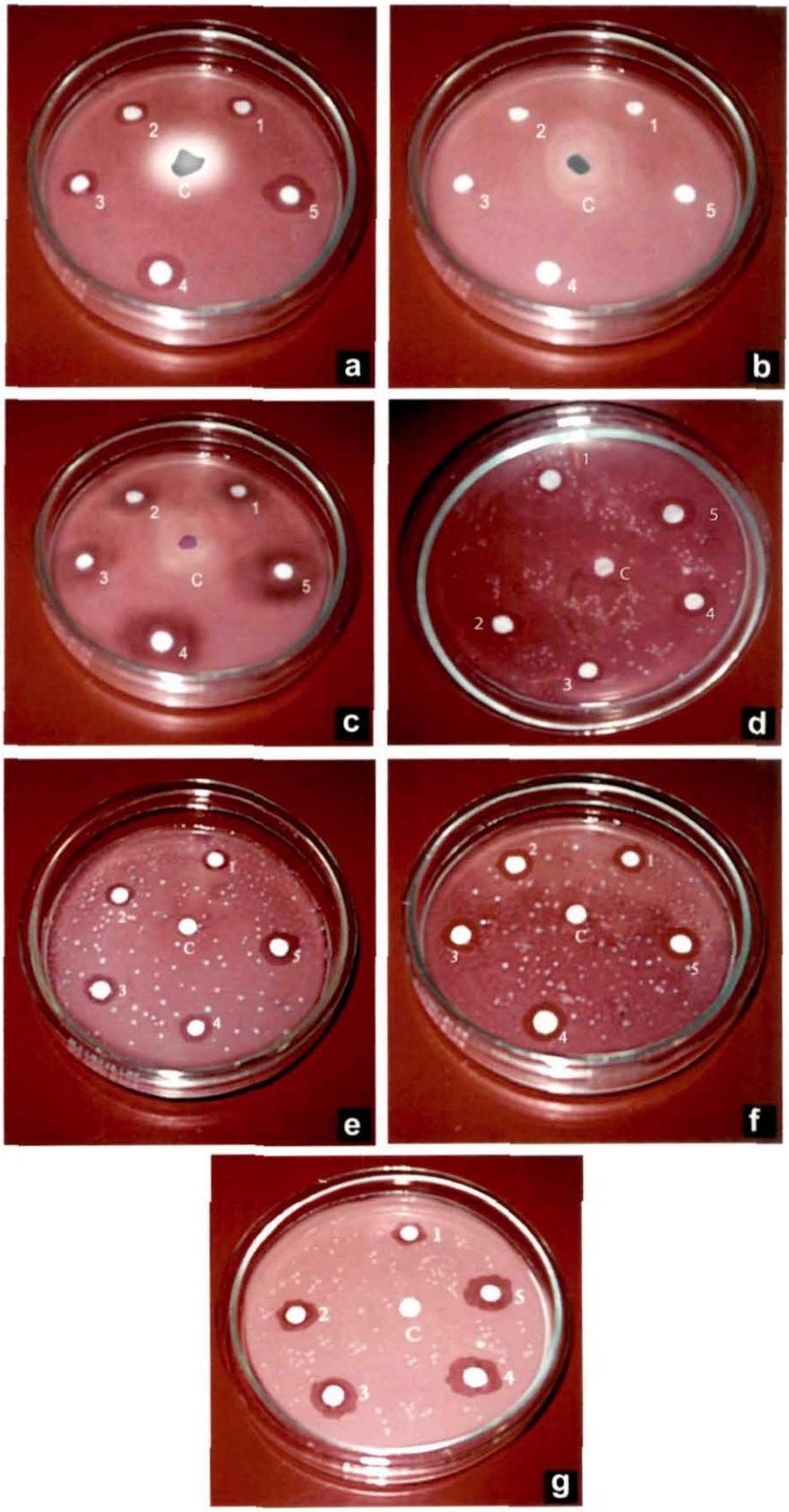


Plate 5: Disc diffusion test for anti microbial activity of compound E: (Lupane-[2,3-C]-**1′,2′,5′**-oxadiazole); at five different concentrations* against (a) *Colletotrichum gloeosporioides* (b) *Curvularia eragrostidis* (c) *Fusarium equiseti* (d) *Ralstonia solanacearum* (e) *Pseudomonas syringae* (f) *Erwinia carotovora*. (g) *Xanthomonas* sp.

* 1=100ppm, 2=200 ppm, 3=300 ppm, 4=400 ppm, 5=500ppm, c=DMSO control.

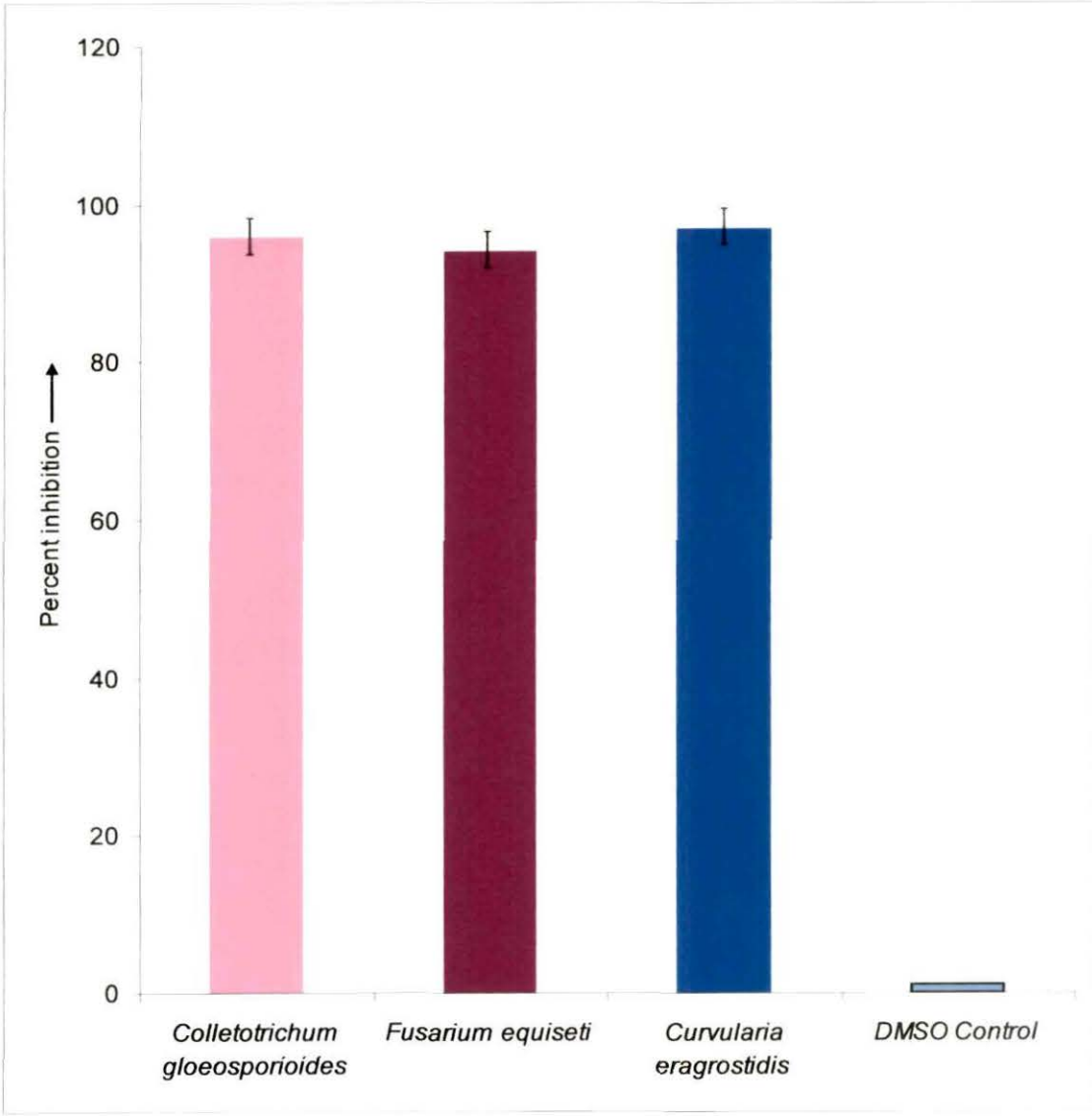


Fig. 22: Percent inhibition of spore germination at 300 ppm concentration of compound E (Lup [2,3-c]-1′,2′,5′-oxadiazole).

maintained in the experimental garden of the Department of Botany with normal watering. Sixty such pots containing plants (each 10 cm in height) were kept in a net house. The plants were grouped into six sets and each set contained 10 plants each. Five compounds (at 100 ppm concentration) were sprayed in five sets separately and the sixth set was sprayed with sterile distilled water. The plants were observed upto seven days. No phytotoxicity was observed at 100ppm concentration of the compounds when compared with the plants of control set which were sprayed with the sterile distilled water. (table-2.22)

Table 2.22: Phytotoxicity of lupeol and its four derivatives

Compounds	Concentrations (ppm)	Morphological & Physiological abnormalities abnormalities			
		1day	3day	5day	7day
A	100	Plants alive, no significant changes Root-germinated Shoot- germinated	Plants alive, no significant changes Root-germinated Shoot- germinated	Plants alive, no significant changes Root-germinated Shoot- germinated	Plants alive, no significant changes Root-germinated Shoot- germinated
B	100	Plants alive, no significant changes Root-germinated Shoot- germinated	Plants alive, no significant changes Root-germinated Shoot- germinated	Plants alive, no significant changes Root-germinated Shoot- germinated	Plants alive, no significant changes Root-germinated Shoot- germinated
C	100	Plants alive, no significant changes Root-germinated Shoot- germinated	Plants alive, no significant changes Root-germinated Shoot- germinated	Plants alive, no significant changes Root-germinated Shoot- germinated	Plants alive, no significant changes Root-germinated Shoot- germinated
D	100	Plants alive, no significant changes Root-germinated Shoot- germinated	Plants alive, no significant changes Root-germinated Shoot- germinated	Plants alive, no significant changes Root-germinated Shoot- germinated	Plants alive, no significant changes Root-germinated Shoot- germinated
E	100	Plants alive, no significant changes Root-germinated Shoot- germinated	Plants alive, no significant changes Root-germinated Shoot- germinated	Plants alive, no significant changes Root-germinated Shoot- germinated	Plants alive, no significant changes Root-germinated Shoot- germinated
Control	Sterile distilled water	Plants alive, no significant changes Root-germinated Shoot- germinated	Plants alive, no significant changes Root-germinated Shoot- germinated	Plants alive, no significant changes Root-germinated Shoot- germinated	Plants alive, no significant changes Root-germinated Shoot- germinated

2.3: DISCUSSION

The present study was based on the phytochemical investigations and antimicrobial activities of the plant-*Xanthoxylum budrunga.* This plant contains medicinal as well as anti-microbial properties. Lupeol, a triterpenoid (special group of secondary metabolites) was the mother compound isolated from the plant.

Triterpenoid lupeol containing a C30 skeleton was isolated and characterized from *X. budrunga* plant of proven anti-inflammatory, anti-tumor and antimicrobial activity.[5,13] Saleem *et al.*(2001) reported that Lupeol, a triterpene, inhibits early responses of tumor promotion induced by benzoyl peroxide in murine skin. Malini *et al.*(2000) also reported that protective effect of triterpenes on calcium oxalate crystal induced peroxidative changes in experimental urolithiasis.

The stem bark of the plant was used to treat cough, cold, toothache, infected wounds, chest pain etc. [3] Fernandez *et al.* (2001) revealed a new insights into the mechanism of action of the anti-inflammatory triterpene lupeol. Yadava and Chakravarti (2008) reported anti-inflammatory activity of a new triterpenoid saponin from *Carthamus tinctorius* whose mechanism of action was similar to lupeol. Commercially the plant is used to make heavy flooring, furniture, agricultural instruments *etc*[4]. The medicinal properties deserve more attention because of the claimed antimicrobial activities which have been confirmed by pharmacological research [7].

In the present study four derivatives of isolated triterpenoid (lupeol) were prepared and characterized. Bioassays of all the five natural or derived compounds were performed against some fungal and bacterial pathogens of economically important plants. Lupeol (compound A), the mother compound was extracted from dried and powdered trunk bark of the plant *Xanthoxylum budrunga* with chloroform in Soxhlet apparatus for 20 hours. In these extraction process different compounds were obtained through column chromatographic separation. From the fractions obtained lupeol was identified and characterized. From lupeol, an intermediate derivative called lupanol was prepared by hydrogenation process and were characterized. Another intermediate derivative lupanone was prepared from lupanol by Jone's oxidation process. In Jone's oxidation different fractions were obtained from column chromatographic separation and the derivatives

were characterized. Lupanone treated with N-Bromosuccinimide form 2,2-dibromolupanone (compound B) and 2α-bromolupanone (compound C). Both compound-B and compound-C were characterized. Another derivative of lupeol named lup-1(2)-en-3-one (compound D) was prepared by dehydrobromination of 2α-bromolupanone with lithium bromide–N,N-dimethyl formamide and was also characterized. Lupan-[2,3-C]-1́,2́,5́-oxadiozole, called compound-E, the fourth derivative of lupeol was prepared by cyclisation of 2,3-dioximelupane in dry DMF under microwave irradiation (100w,100^{0}C) for 10 mins.

To observe antimicrobial activity of lupeol and its derivatives three fungi *viz. Colletotrichum gloeosporioides, Fusarium equiseti* and *Curvularia eragrostidis* and four bacteria *viz. Ralstonia solanacearum, Xanthomonas sp., Pseudomonas syringae* and *Erwinia carotovora* were used in the present study. All the five chemicals were tested for their phytotoxicity, if any, on tomato plants.

The biological activity of natural lupane derivatives was reliably established [34]. In the last decade, interests in the pharmacological properties of lupane derivatives were observed after revealing promising antiviral (particular Anti-HIV) and antineoplastic agents among these compounds [35]. Antifungal and antimicrobial activities of lupeol was also reported [16].

The compound–A, chemical name 'Lupeol' showed antibacterial activity and anti fungal activity.. In disc diffusion studies growth of two fungi (*Colletotrichum gloeosporioids, Fusarium equiseti)* were controlled by compound-A. *Curvularia eragrostidis* could not be controlled in disc diffusion test even at 500ppm concentration of lupeol. Lupeol showed little activity against *Ralstonia solanacearum* at lowest concentration (100ppm). In contrast, three other bacteria (*Xanthomonas sp, P. syringae, E. carotovora*) tested were controlled significantly at 100ppm concentration of lupeol.

Compound–B (2,2dibromolupanone) a derivative of compound-A was stronger than the mother compound Lupeol, as evident from the result of spore germination bioassay and disc diffusion bioassay against three different fungi (*Colletotrichum gloeosporioids, Fusarium equiseti* and *Curvularia eragrostidis).* In contrast to the mother compound (lupeol) which could not check the growth of *Curvularia eragrostidis* in disc

diffusion test, compound-B could control *Curvularia eragrostidis* at all the five concentrations (100, 200, 300, 400 & 500ppm) tested. Stronger antibacterial activity of compound-B was also evident fron the disc diffusion test against the four bacterial pathogens (*R.solanacearum, Xanthomonas sp., P.syringae* and *E.carotovora*).

The second derivative of lupeol coded as compound-C (2α-bromolupanone) was tested for antifungal and antibacterial activity. Compound-C could check spore germination of all the three fungi (*Colletotrichum gloeosporioides, Fusarium equiseti* and *Curvularia eragrostidis*) moderately at five different concentrations of the compound tested. In disc diffusion test the compound showed no activity against *Curvularia eragrostidis*. The compound showed poor activity against *Colletotrichum gloeosporioides* but showed high activity against *Fusarium equiseti.* In case of bacteria only *E. carotovora* was checked at 100ppm concentration. Growth of all three other bacteria (*Ralstonia solanacearum, Xanthomonas sp.& Pseudomonas syringae*) were controlled only at higher concentrations of the compound-C. Thus compound-C was less antimicrobial than compound-B.

The third derivative of lupeol presently coded as compound-D (lup-1(2)-en-3-one) showed moderate antifungal activity both in case of spore germination bioassay and disc diffusion bioassay. In higher concentrations of the compound all the four bacteria (*R. solanacerum, Xanthomonas sp., P. syringae* and *E. carotovora*) were controlled significantly as evident from the result presented in table-2.16. In lower concentrations of compound-D, *Xanthomonas* sp and *E. carotovora* were controlled but it could not control growth of *R. solanacerum* and *P. syringae.*

The fourth derivative of lupeol coded as compound-E (Lupane[2,3-C]-1′,2′,5′-oxadiozole) was also subjected to spore germination bioassay and the results (presented in table-2.19) showed good antifungal activity when tested in spore germination bioassay. Inhibition of spore germination ranged between 80-100% in the spore germination bioassay. In disc diffusion bioassay 100ppm and 200ppm concentration of compound-E showed no antibacterial inhibition zone on the plates. However, higher concentrations of compound-E could control the three bacteria significantly even at lowest concentration tested (100ppm). All the four bacteria showed moderate to high antibacterial activity

respectively with the increasing concentrations (100ppm-500ppm) of the compound-E. Thus the compound showed good antibacterial activity and moderate antifungal activity.

Similar studies were also done by Shai *et al.* (2008) who isolated four compounds lupeol (1), betulinic acid (2), ursolic acid (3) and 2-alpha-hydroxyursolic acid (4) from the leaves of *Curtisia dentata*. They studied the antibacterial and antifungal activity using broth microdilution assay and bioautography method. Mansouri (1999) found new antibacterial agents from ethanolic extracts of ten plants. The extracted compounds from the plants were screened for antibacterial activity. The agents were effective against *Staphylococcus aureus*. Among the plants *Mentha vividis L.* was also active against the isolates and inhibited the growth of 48.7% of the isolates. Kamalakanman *et al.* (2009) extracted 20 plant leaves and screened their inhibitory effect against the rice blast pathogen. They reported that *Prosopis juliflora* followed by *Zizyphus jujube* and *Abutilon indicum* significantly inhibited the mycelial growth and biomass as well as toxin production and spore germination under laboratory conditions. Ragasa *et al.* (2001) extracted the air dried leaves of *Vitex negundo* which afforded vitexilactone and casticin by silica gel chromatography. Their structures were elucidated by extensive 1D and 2D NMR spectroscopy. They studied their activity and found antifungal activity (against *Candida albicans* and *Aspergillus niger*) and antibacterial activity (against *Staphyllococcus aureus* and *Pseudomonas aeruginasa*). They also reported inactivity of the compounds against *Escherichia coli* and *Bacillus subtilis*. Hence, it was necessary to screen antimicrobial activity of the basic triterpenoid compound and its derivatives. From our studies, thus, antimicrobial activity of lupeol and its derivatives against some fungus and bacteria were significantly screened. Saleh *et al.* (1999) also isolated the known triterpenoids lantic acid, camarinic acid and lantanilic acid from *Lantana camara* (L) cultivated in Egypt and carried out the antibacterial activity of lantic acid using bioautography assays for Gram-positive and Gram-negative bacteria. They also compared their doses to 0.05 and 0.005 μg for chloramphenicol. They showed that lantic acid has broad spectrum antibacterial activity and may hold potential as a non-selective antimicrobial agent. Khan *et al.* (2008) isolated amblyone, a triterpenoid from *Amorphophallus campanulatus* and studied *in vitro* antibacterial, antifungal and cytotoxic

activities using disc diffusion technique. The minimum inhibitory concentration of amblyone was determined using serial dilution technique. They observed large zones of inhibition in disc diffusion test. They performed antibacterial screening against four Gram-positive bacteria (*Bacillus subtilis, Bacillus megaterium, Staphylococcus aureus* and *Streptococcus pyogenes*) and six Gram-negative bacteria (*Escherichia coli, Shigella dysenteriae, Shigella sonnei, Shigella flexneri, Pseudomonas aeruginosa* and *Salmonella typhi*). The MIC values determined against those bacteria were ranged between 8 to 64 µg/ml. In antifungal screening, the compound showed small zones of inhibition against *Aspergillus flavus, Aspergillus niger, Rhizopus aryzae*, and *Candida albicans*. Thus fungi were resistant against the compound. Similarly, our results also showed the susceptibility and resistance of the pathogens against the six compounds tested. The effective and nonphytotoxic concentration (100 ppm) was comparatively low than the concentrations tested by other authors. Thus the significance of the study was established.

On the basis of phytotoxicity tests, it was found that 100ppm concentration of compounds A, B, C, D, and E were not phytotoxic. From the present study, it was evident that several plant pathogens could be controlled by the compounds at 100 ppm concentration (non phytotoxic). Hence, the effective compounds may be recommended for controlling the respective plant pathogens *in vivo* or in field conditions.

Section 3: References

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■ CHAPTER - III

Title: Extraction and characterization of Betulinic acid from *Bischofia javanica*, preparation of its derivatives and antimicrobial activity of each of them

Several medicines have been extracted from plants since pre-historic ages. Certain alkaloids, steroids, terpenoids, and the secondary metabolites functioned as defensive agents against harmful pathogens [1]. It was also assumed that these compounds were used to treat human aliments. Following this, people from all over the world made some attempts to isolate these classes of natural products from the medicinal plants and evaluated their biological activities [2]. In the present chapter, the antimicrobial properties of the plant *Bischofia Javanica* have been discussed.

Bischofia javanica is an evergreen or semi evergreen woody tree with a maximum height of 40 meter and diameter of 2.3 meter. The relative short trunk is erect, branches low. The bark in nearly smooth, grayish brown, 1 cm thick and contains a red milky sap that become resinous and semi solid when dried. The male inflorescence is 8-13 cm long and pubescent to glabrous, while the female inflorescence is 15-17 cm long and pendent. Appearing in August to October, berry like fruits are light brown, globular or sub globular 6-13 cm in diameter, containing oblong seeds 5mm in length [5]. The plant *Bischofia javanica* belongs to the family Euphorbiaceae. *Bischofia* is a very small member of the family Euphorbiaceae and contain only two species distributed from southern Asia to Australia. They also occur in south-western, central and eastern part of China. In India it is found only in north Himalayan region [3].

The wood of *Bischofia javanica* is red, heavy, hard, and fine grained and used as a material for buildings. The fruits are used in making wine. Containing 30-45% oil, the edible seed is a source of red dye. The roots are used for medicinal properties. It also showed antifungal activities [4].

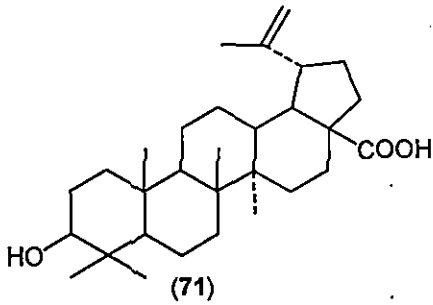
The ethanol extract of the bark of *Bischofia javanica* was partitioned in petroleum ether and dichloromethane then with ethyl acetate. The crude ethnolic extract and several

partitioned fractions showed broad spectrum anti-bacterial activities [6]. Betulinic acid and its derivatives were isolated from Bioassay guided fractionations of the $CHCl_3$ extract of the outer bark of *Bischofia javanica*.

Section 1: Extraction and characterization of pentacyclic triterpenoid (Betulinic acid) from *Bischofia javanica*.

1.1. Extraction from outer bark of *Bischofia javanica*: Isolation of Betulinic acid.

Dried and powdered trunk bark (3 kg) of *Bischofia javanica* was extracted with ethylacetate in Soxhlet apparatus for 72 hours. Ethyl acetate was distilled off and the gummy residue (14 g) was taken up in ether (1.0 lt). The ether solution was washed with 10% aqueous sodium hydroxide solution (3x300 mL). The aqueous alkaline layer was thoroughly shaken with ether to remove any neutral material that might be present. The aqueous layer was acidified (1 lt) when some insoluble solids separated out. The acidified portion was extracted with ether, washed with water until neutral and then dried using anhydrous Na_2SO_4. Ether was removed when a gummy residue (10 g) of crude betulinic acid was obtained and purified by column chromatography. Suitable mixture of petroleum ether and ethylacetate were used as eluent and crystallized from chloroform-methanol afforded betulinic acid of m.p 299^0—302^0C.



(71)
Betulinic acid

1.2. Estarification of Betulinic acid: Isolation of Methylbetulinate

To the crude betulinic acid (10 g) dissolved in ether (600 mL), a solution of diazomethane in ether [prepared from *N*-nitrosomethyl urea (5 g)] was added at cold and

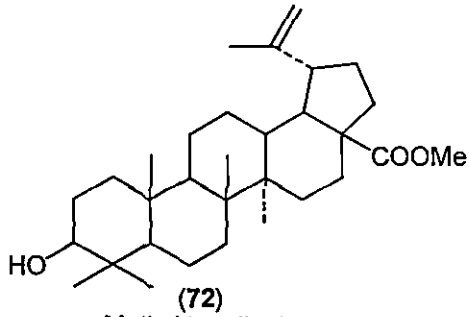
the reaction mixture was kept overnight. Next day excess of diazomethane was destroyed with glacial acetic acid (2 mL) .The ether solution was washed with water, then 10% sodium bicarbonate solution and again with water until neutral and dried by using anhydrous Na_2SO_4. Evaporation of the ether yielded a gummy residue (4.5 g). This crude ester dissolved in petroleum ether (15mL) was placed over a column of silica gel (60-120 mesh, 30 g) developed with petroleum ether and was eluted with the following solvents (table3.1).

Table 3.1: Examination of different fractions obtained from column chromatographic separation

Eluent	Fractions 50 mL each	Residue on evaporation	Melting point
Petroleum ether	1-4	oil	-
Petroleum ether: ethyl acetate (4:1)	5-8	nil	-
Petroleum ether: ethyl acetate (3:2)	9-14	solid	222^0-224^0C

Further elution with more polar solvent did not afford any solid material

1.3: Examination and characterization of Fractions: Same solids obtained from the reactions (Table-3.1) were combined (4 g) and crystallized from a mixture of chloroform-methanol to afford colorless needle shaped crystals of methylbetulinate of m.p. 223^0-224^0C, $[\alpha]_D$ +5.0. The compound was found identical with an authentic sample (mixed m.p, co-IR and co-tlc *etc.*). [Lit.70 m.p 224^0C-225^0C, $[\alpha]_D$ +5.0]



(72)
Methyl betulinate

Analysis report	%C	%H
Present compound	78.79	10.52
Calculated for $C_{30}H_{50}O_3$	79.10	10.71

IR: ν_{Nujol}^{max} 3540 (-OH),1730 (-$COOCH_3$)
1660, 890 cm^{-1} (=CH_2)

1.4. Preparation of Bromo derivatives of Betulinic acid:

Following steps have been carried out to prepare the bromo derivatives:

1.4.1. Hydrogenation of Methyl betulinate:preparation of Methyl dihydro-betulinate

Methyl betulinate (4 g) dissolved in ethyl acetate (250 mL) was shaken in an atmosphere of hydrogen in presence of palladized charcoal (10%) catalysts (50 mg) for three hours until absorption of hydrogen ceased. Ethyl acetate was removed by distillation after filtering off the catalysts. The solution was diluted with water whereby a white solid (3.5 g) separated out which was collected by filtration. Crystallization from a mixture of chloroform-methanol furnished colorless needles of a compound, m.p 236^0-238^{0}C, $[\alpha]_D$ -17.0. This compound was found to be identical with an authentic sample of methyl dihydro betulinate (mixed m.p, co-IR and co-tlc).

IR: ν_{Nujol}^{max} 3540 (-OH), 1705 cm^{-1} (-CO_2Me)

1.4.2. Jone's oxidation of lupanol: Preparation of Methyldihydrobetulinate.

To a solution of methyl dihydro betulinate (3.3 g) in pure acetone (500 mL), Jone's reagent was added drop wise and with continuous shaking until a faint orange colour persisted. The mixture was kept at room temperature for 1 hour, then diluted with water and extracted with ether. The ether layer was washed thoroughly with water, dried by using anhydrous Na_2SO_4 and the solvent was evaporated. The residue (2.9 g) dissolved in minimum volume of petroleum ether was chromatographed over a column of silica gel (20 g). The chromatogram was developed with petroleum-ether and then eluted with the following solvents (Table3.2).

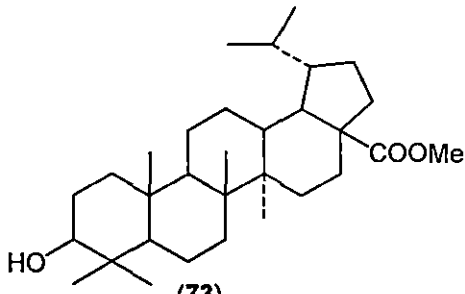
Table 3.2: Examination of different fractions obtained from column chromatographic separation

Eluent	Fractions 50ml each	Residue on evaporation	Melting' point
Petroleum ether	1-4	oil	-
Petroleum ether: ethyl acetate (4:1)	5-20	solid	197^0-199^{0}C

Further elution with more polar solvent did not afford any solid material

Fractions 5-20 (Table-3.2) were combined and crystallized from methanol furnished some needle shaped crystals, m.p. 192^{0}C-193^{0}C, $[\alpha]_D$ +18°, identical with an authentic sample of methyl dihydro betulonate (mixed m.p, co-IR and co-tlc).

[Lit 71 m.p.194^{0}C, $[\alpha]_D$ + 18.4°]



(73)

Methyl dihydrobetulinate

Analysis report	%C	%H
Present compound	79.32	10.86
Calculated for $C_{31}H_{50}O_3$	79.10	10.71

IR: ν_{Nujol}^{max} 1730 (-CO_2Me), 1708 cm^{-1} (C=O)

1.4.3: Treatment of Methyl dihydrobetulonate with N-bromo succinimide: formation of 2,2-dibromo methyl dihydrobetulonate (Compound F) and 2α-bromo methyl dihydrobetulonate (Compound G):

A solution of methyl dihydrobetulonate (2.5 g) in chloroform (125 mL) was mixed with dimethyl sulphoxide (65 mL) N-bromosuccinimide (3.0 g) and added in small lots in order to keep the temperature of the reaction mixture below 25^{0}C and the mixture kept in dark for 10 days. The residue (2.3 g) obtained after usual work up showed two spots on TLC plate, thus existence of at least two compounds were indicative. The residue (2.3 g) was chromatographed over a column of silica gel (20 g). The chromatogram was developed with petroleum ether and eluted with the following solvents (Table 3.3).

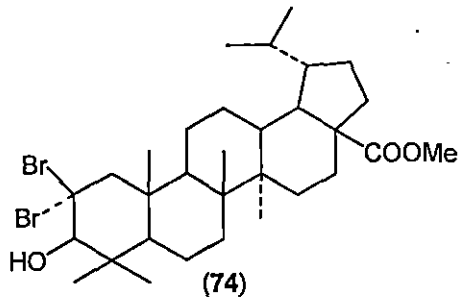
Table 3.3: Examination of different fractions obtained from column chromatographic separation

Eluent	Fractions 50ml each	Residue on evaporation	Melting point
Petroleum ether	1-3	nil	-
Petroleum ether: ethyl acetate (4:1)	4-9	White solid	158^0-159^0C
Petroleum ether :ethyl acetate (4:1)	10	nil	-
Petroleum ether: ethyl acetate (4:1)	11-18	White solid	120^0-122^0C

Further elution with more polar solvent did not afford any solid material

1.4.4: Examination of fraction 4-9 (Table 3.3): Isolation of 2,2-dibromomethyl dihydrobetulonate (compound F):

The fractions 4-9 (Table 3.3) showed homogeneity on TLC plate were combined (0.9 g) and crystallized from of mixture of chloroform-methanol afforded 2,2-dibromomethylcdihydrobetulonate same as compound 'F', m.p 160^0-162^0C. It gave positive Beilstein test for halogens.



(74)

2,2-Dibromomethyl dihydrobetulinate

Analysis report	%C	%H
Present compound	58.82	12.72
Calculated for $C_{31}H_{48}O_3Br_2$	59.05	12.90

UV: 219 nm (e = 7879)

IR: ν_{Nujol}^{max} 1725 cm^{-1} (-CO_2Me), 1705 cm^{-1} (C=O)

1H NMR ($CDCl_3$): 0.76 to 1.22 ppm for 7 methyls,
3.11 and 3.63 (2d, 1H,1-CH2, J = 16Hz)
3.65 (s, 3H, -$COOCH_3$) ppm

MASS: m/z at 628, 626, 624 (M+), 571, 569, 567, [M-$COOCH_3$], 550, 548, 547[MHBr]+, 533, 531, 525, 523, 468, 470, 471, 453, 412, 411, 410, 409, 283, 285, 274, 231, 205, 203, 177 (base peak)

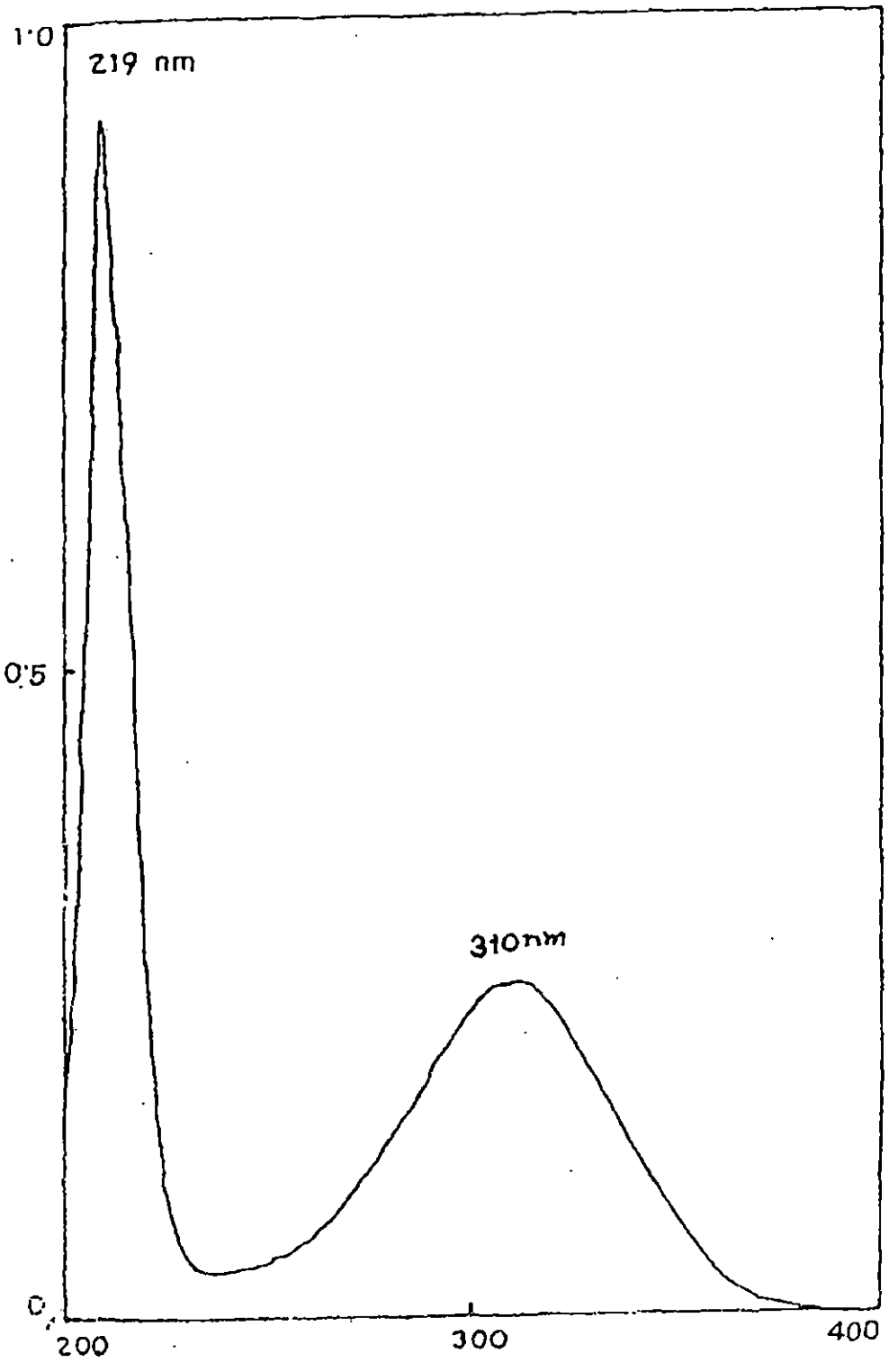


Fig. 23: UV Spectrum of 2,2– .bromo methyl dihydro betulonate (Compound F)

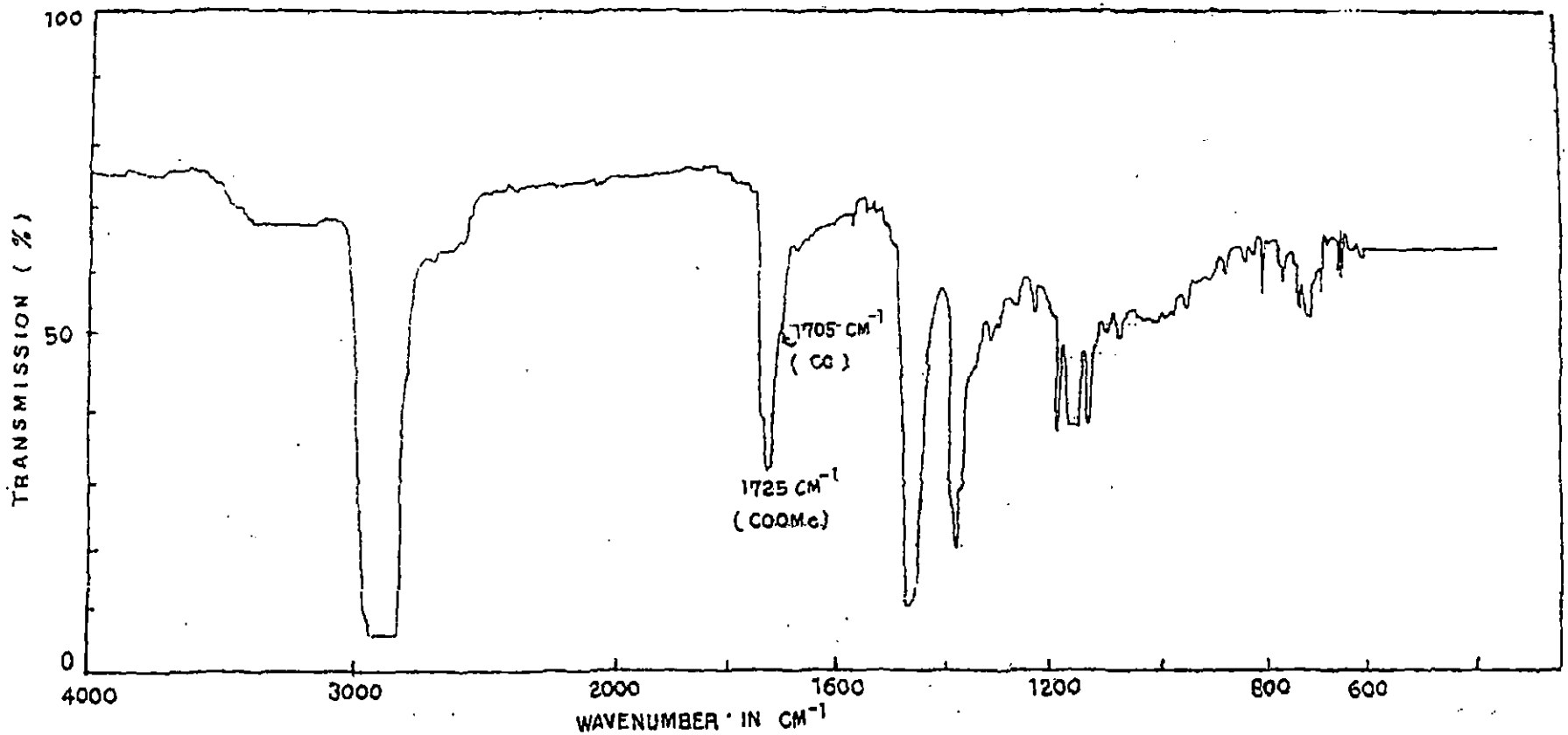


Fig. 24: IR Spectrum of 2,2-dibromo methyl dihydro betulonate (Compound F)

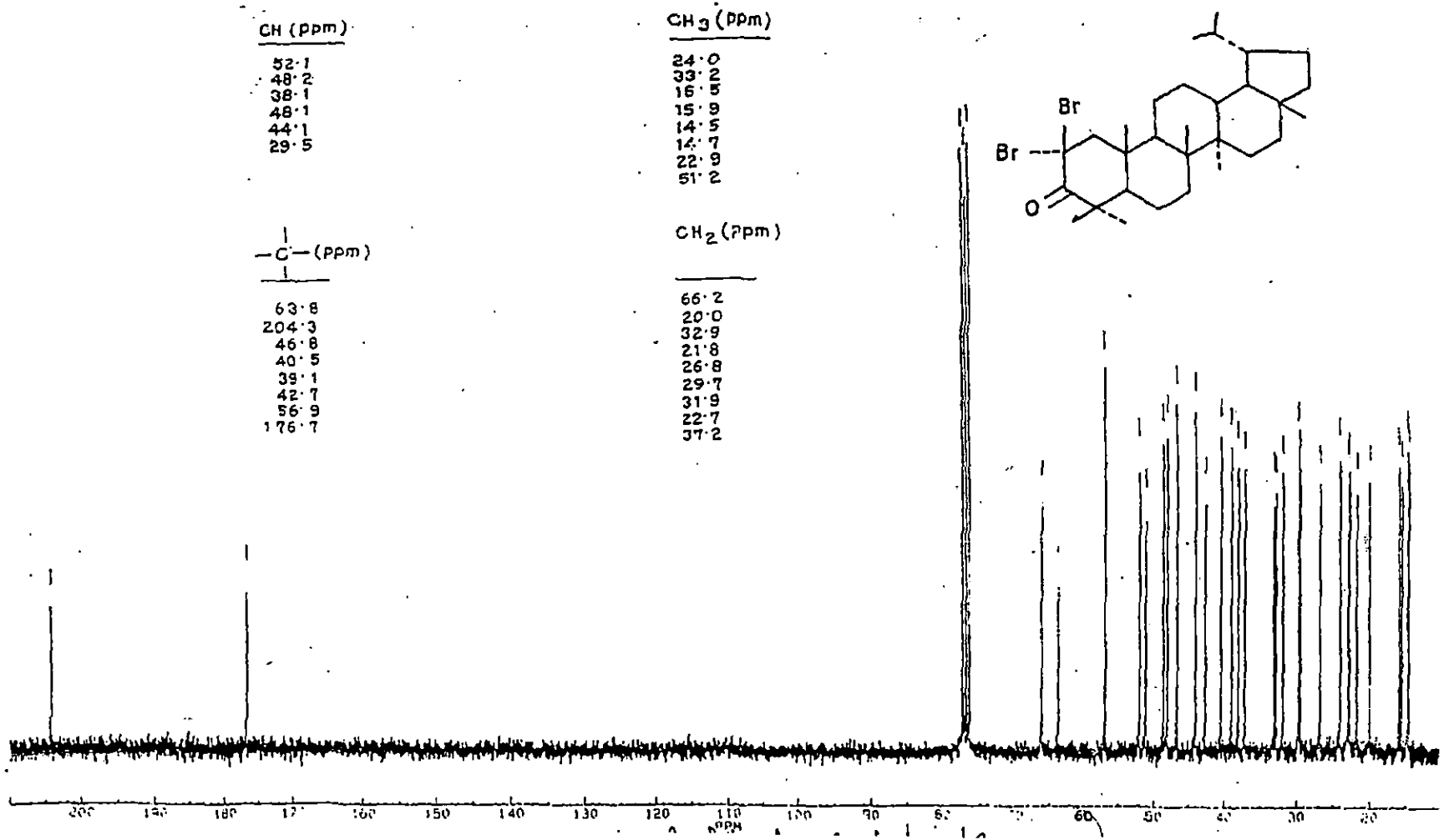
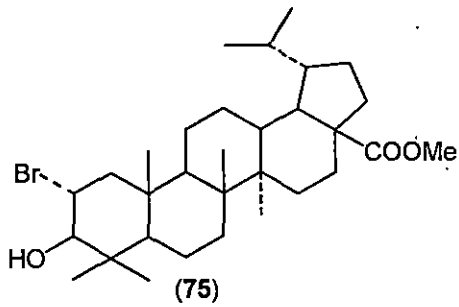


Fig. 25: ^{1}H NMR 2,2-dibromomethyldihydro betulonate (Compound F)

1.4.5: Examination of fractions 11-18 (Table 3.3): Isolation of 2αbromo methyl dihydro betulonate (compound G):

The fractions 11-18 (Table3.3) were combined (1.2 g) and crystallized with chloroform-methanol mixture to afford crystals of 2α-bromo methyldihydrobetulonate same as compound G, m.p 125^0-127^0C responded to belistein test for halogen and was identified as 2α-bromo methyl dihydro betulonate.



(75)
2α–Bromomethyl dihydrobetulonate

Analysis report	%C	%H
Present compound	67.21	13.18
Calculated for $C_{31}H_{49}O_3Br$	67.64	13.77

IR: : ν_{nujol}^{max} 1725 (-COOMe), 1705 cm^{-1}(C=O)

1H NMR ($CDCl_3$): 0.94, 0.97, 1.09,1.13, 1.20
(4s, 12H, 4t-CH_3)
0.77 and 0.87 (2d, 6H, 2s-CH_3, 1-CH J = 7 Hz)
2.65 (dd, 1H, 1-CH, J = 12 Hz and 6 Hz)
2.67 (t, 1H, 1C-H, J = 12 Hz)
5.06 (dd, 1H, 2-CH, J = 12 and 6 Hz) ppm

MASS: m/z at 550, 548(M+) (1:1)+, 491, 489, [M-COOCH3], 471, 470, 469, [M-Br]; 412, 411, (100%), 410, 395, 275, 260, 250, 205, 191, 177, 174, 119 (base peak) .

1.4.6. Dehydro bromination of 2α-bromo dihydro methyl betulonate: Isolation of 28carbomethoxy lup-1(2)-en-3-one (compound-H)

Compound G (200 mg) was refluxed with distilled DMF (30 mL) and lithium bromide (300 mg) for six hours. The residue (16g) obtained after usual work up was dissolved in minimum volume of benzene and chromatographed over a column of silica gel (15 g). The column was developed with petroleum ether and eluted with the following solvents (Table 3.4).

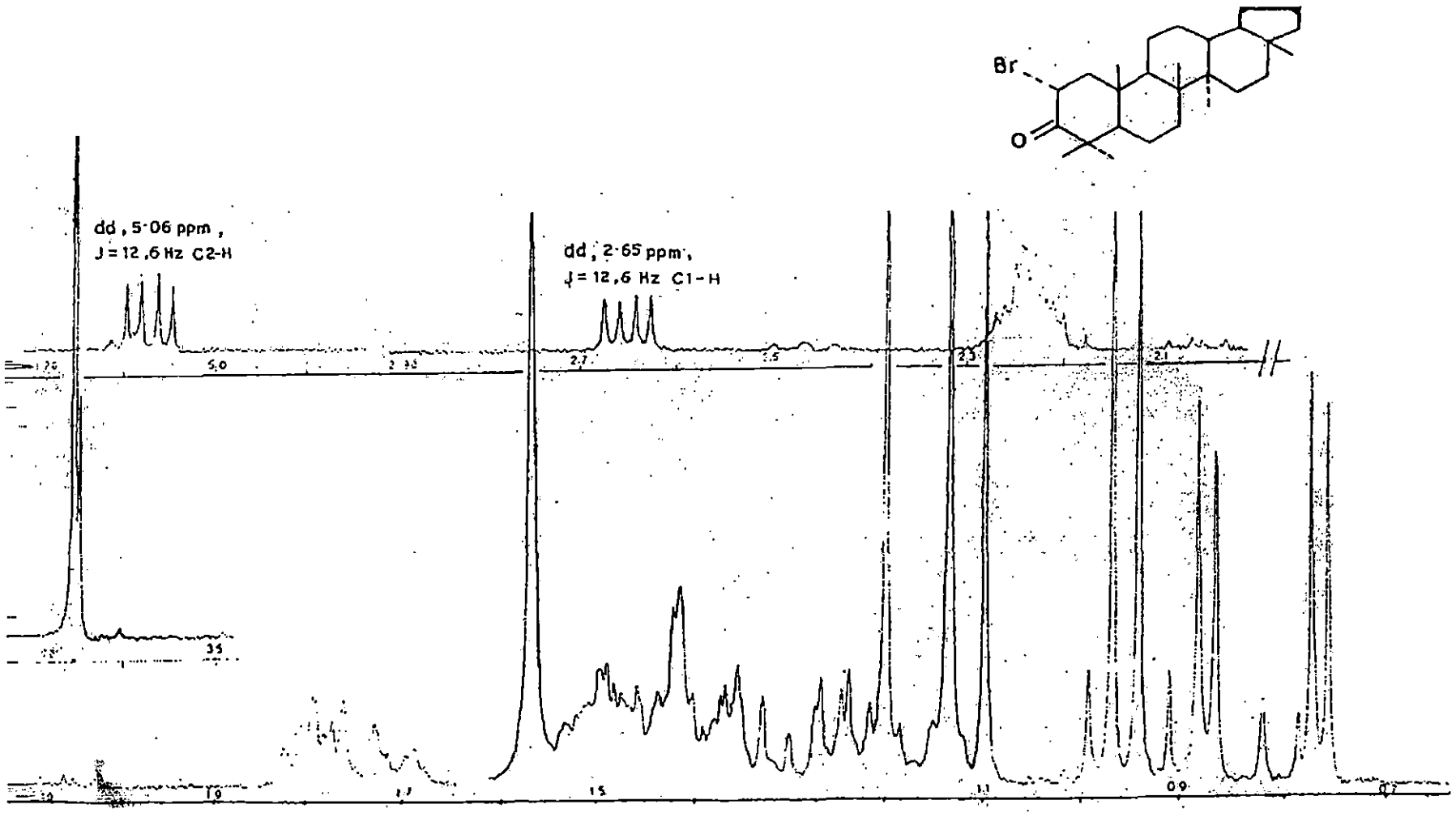


Fig. 26: ^{1}H NMR of 2α– bromo methyl dihydro betulonate (Compound G)

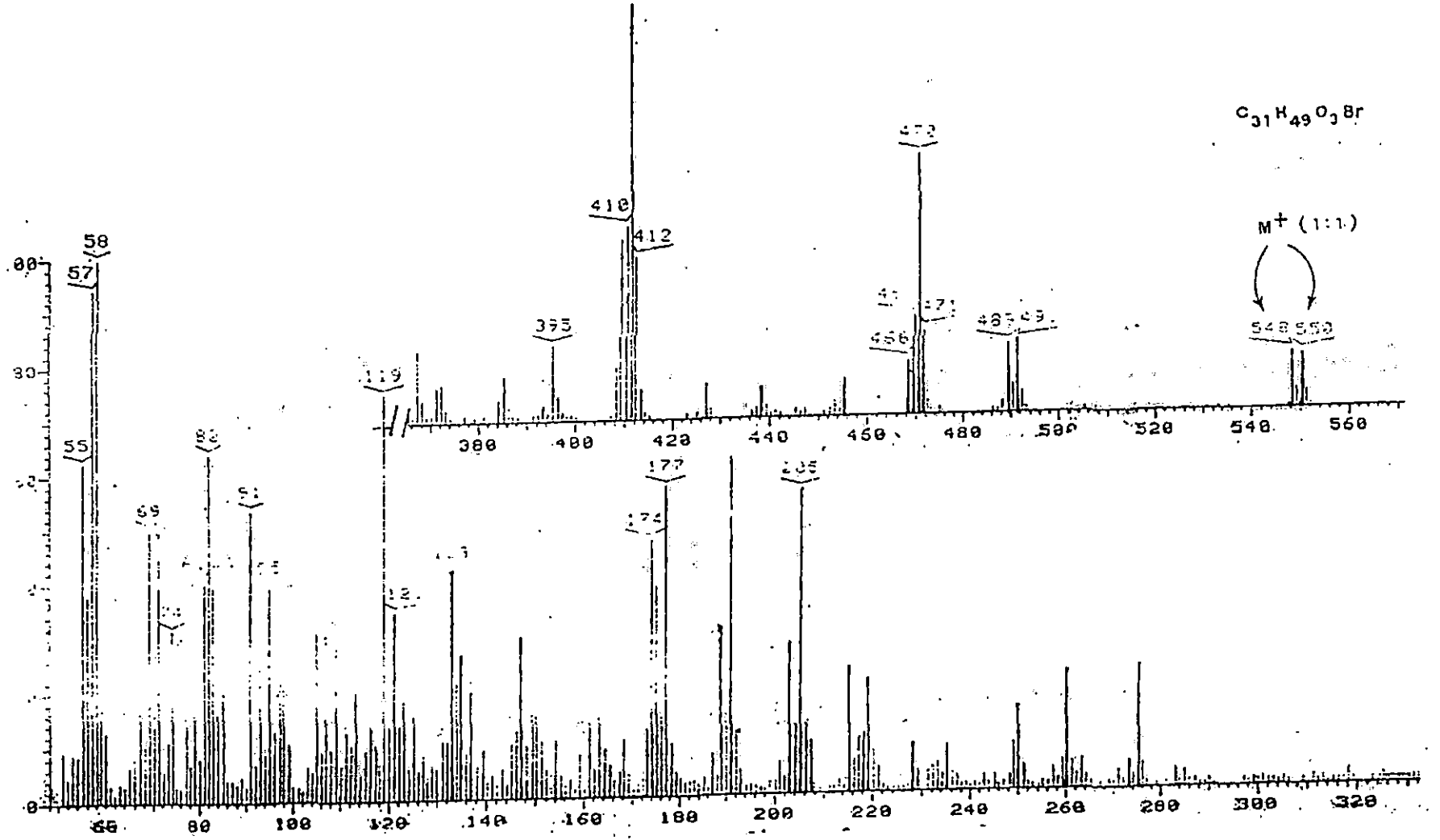


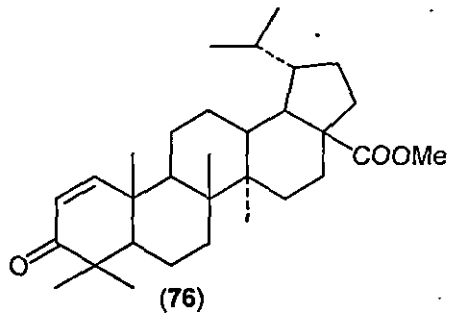
Fig. 27: Mass spectrum of 2α– bromo methyl dihydro betulonate (Compound G)

Table 3.4: Examination of different fractions obtained from column chromatographic separation

Eluent	Fractions 50ml each	Residue on evaporation	Melting point
Petroleum ether	1-3	nil	-
Petroleum ether :ethyl acetate(1:4)	4-14	nil	180^0-183^0C

Further elution with more polar solvent did not afford any solid material

Fractions 4-14 (Table 3.4) showed homogeneity on TLC plate were combined (0.15 g) and crystallized from a mixture of chloroform-methanol. The crystallization furnished colorless needle shaped crystals of compound H, m.p 186^0-187^0C and showed negative test for halogen and was identified as 28-carbomethoxy-lup-1(2)-ene-3-one *same as compound H.*



(76)

28-Carbomethoxy lup-1(2)-3-one

Analysis report	%C	%H
Present compound	79.28	10.24
Calculated for $C_{31}H_{48}O_3$	79.49	10.26

Beilstein test: Positive

UV: λ_{MeOH}^{max} 228 nm (e = 18,000)
IR: : ν_{Nujol}^{max} 1735 cm^{-1} (-COOMe), 1670 cm^{-1} (-C=C-C-)

1H NMR ($CDCl_3$): 0.96, 1.00, 1.07, 1.09, 1.13, (5s,15H 5 t-CH_3), 0.78 and 0.88 (2d,6H,2 S-CH_3, J= 8 Hz), 3.86 (s, 3H, -COO-CH_3) 5.81 and 7.11 (2d, 2H, H-C=C-H, J = 10 Hz) ppm

MASS: m/z at 468 $[M]^+$ 408 [-CH_3COOH]+, 393, 275, 271, 215, 150, 137, 121, 93, 69 (Base peak).

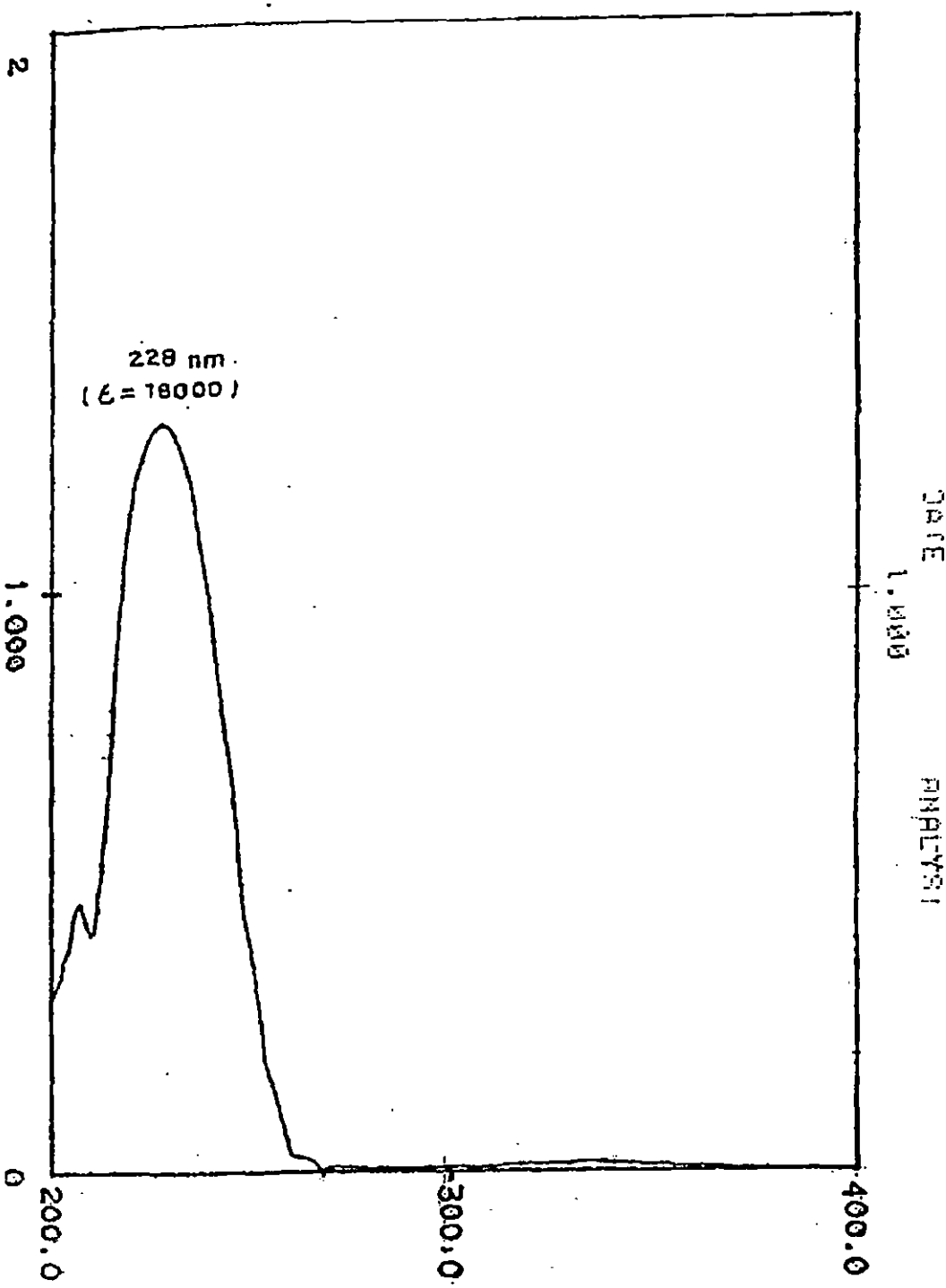


Fig. 28: UV Spectrum of 28-carbomethoxy lup-1-(2)-3-one (Compound H)

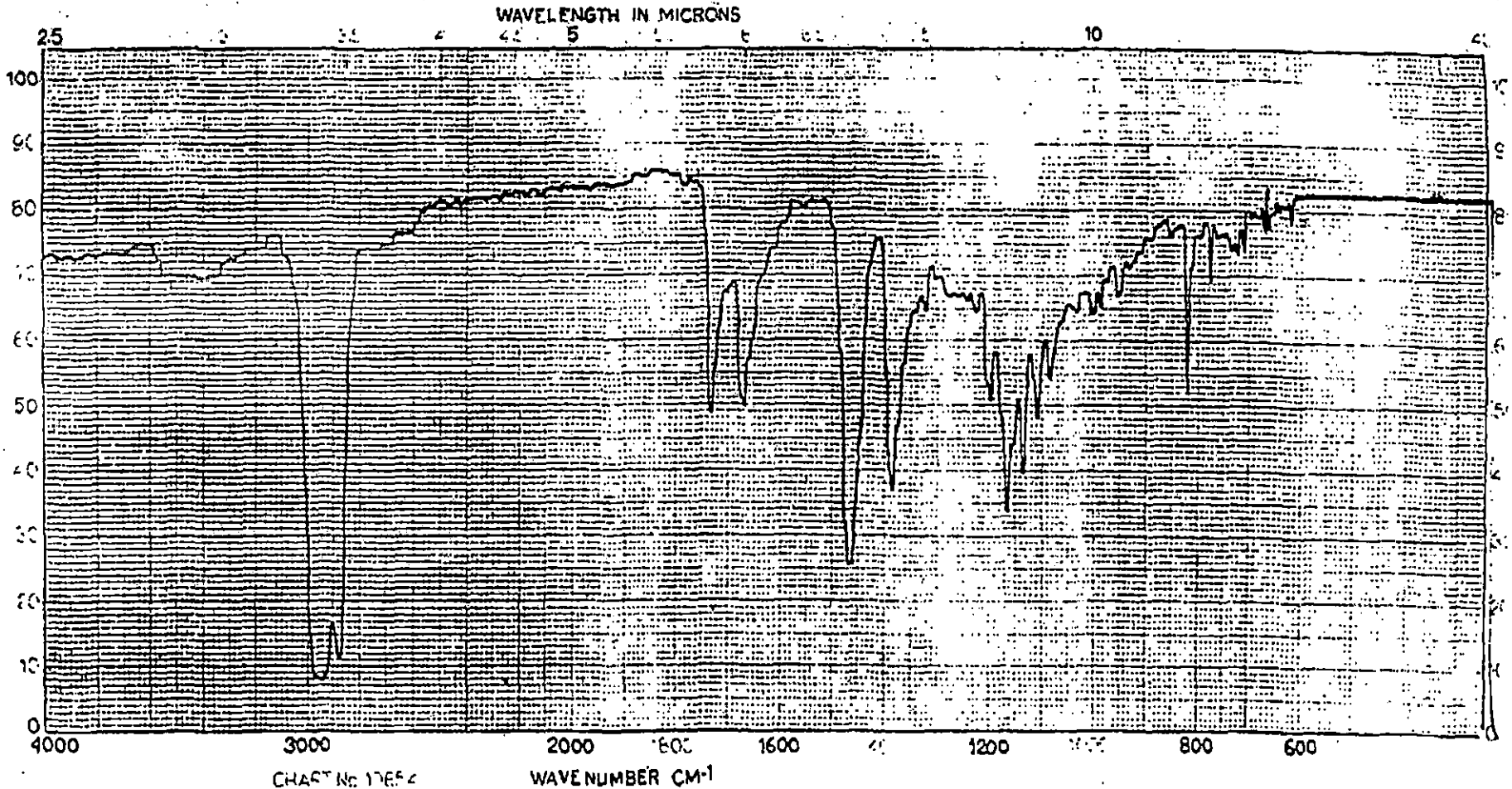


Fig. 29: IR Spectrum of 28-carbomethoxy lup-1-(2)-3-one (Compound H)

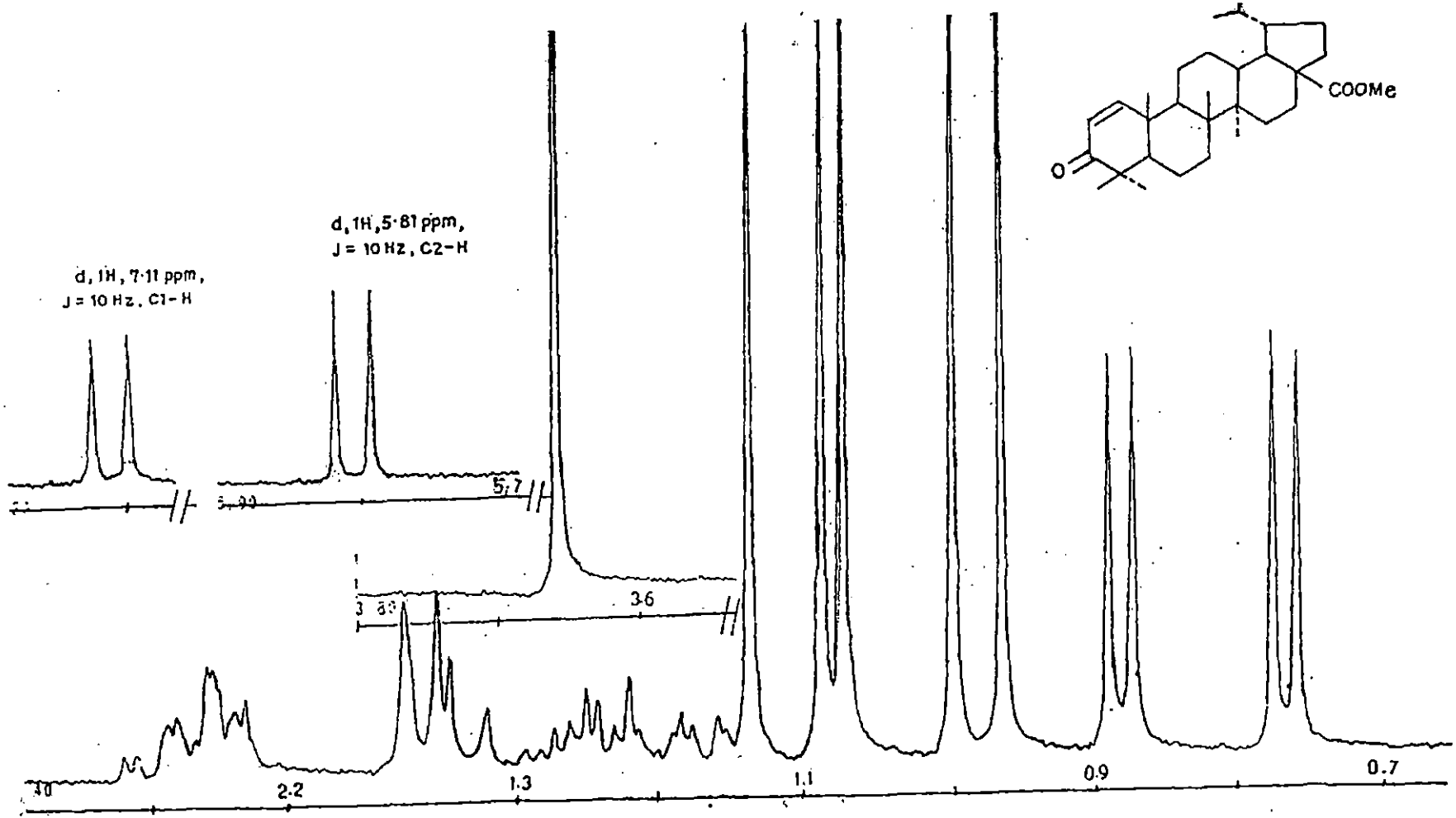


Fig. 30: ^{1}H IR Spectrum of 28-carbomethoxy lup-1-(2)-3-one (Compound H)

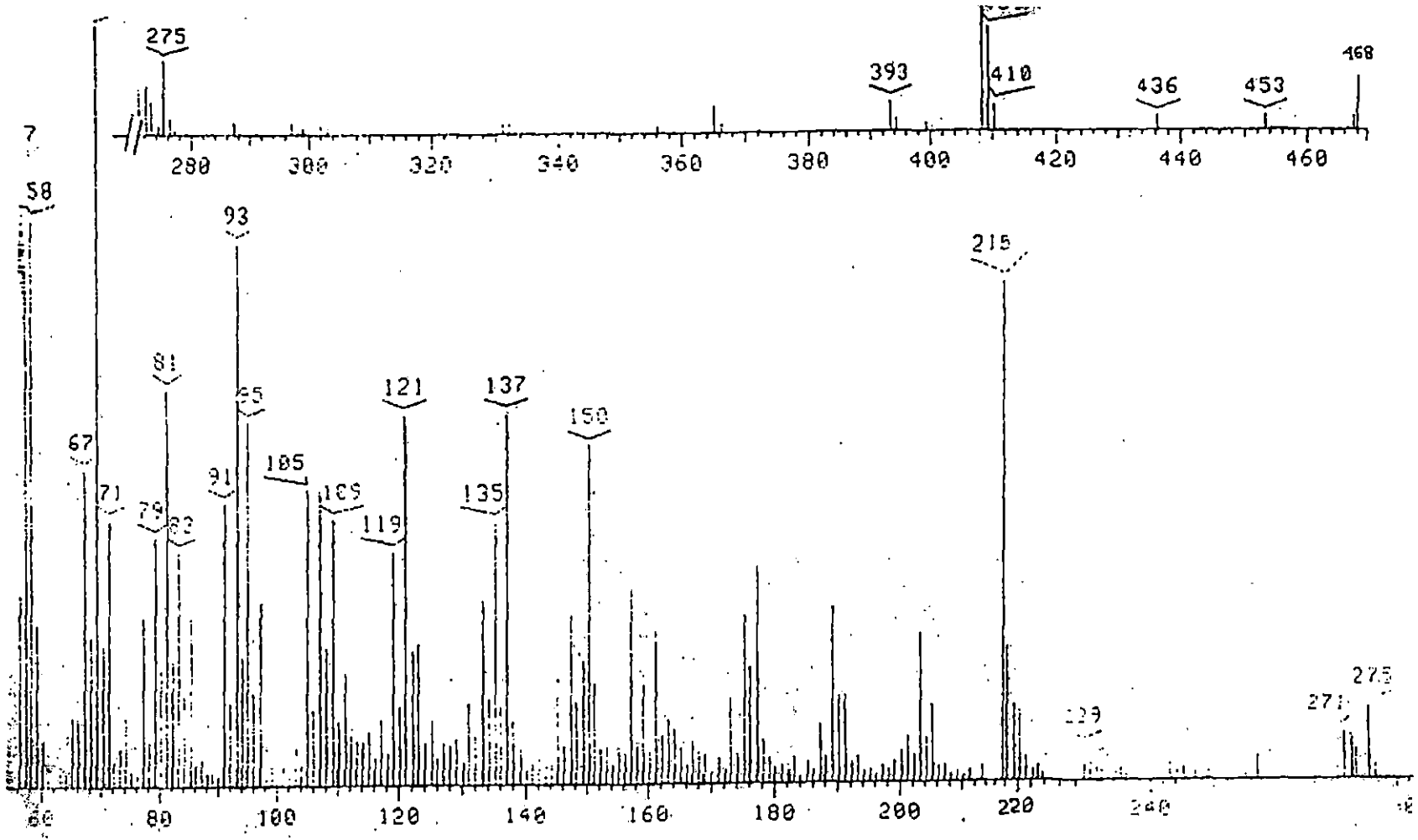
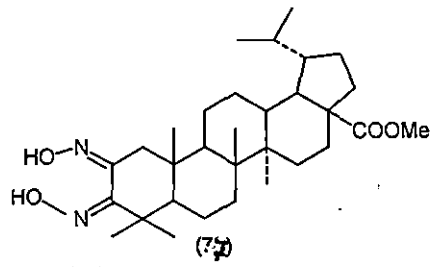


Fig. 31: Mass spectrum of 28-carbomethoxy lup-1-(2)-3-one (Compound H)

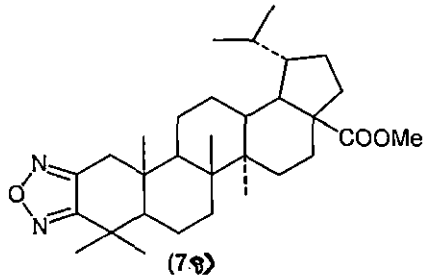
1.4.7. Cyclisation of the dioxime to 28-carbomethoxy lupane[2,3-C]-1′,2′,5′-Oxadiazole (compound - I)

28-Carbomethoxy-lupane[2,3-C]-1′,2′,5′-oxadiazole was prepared by cyclization of 28-carbomethoxy-2,3-dioximinolupane in dry DMF under microwave irradiation (100W, 100^{0}C) for 10 minutes. The residue obtained after usual work up was purified by repeated crystallization by chloroform-methanol mixture and designated as compound 'J', analyzed for $C_{31}H_{48}O_4N_2$ which was corroborated by mass spectral analysis that showed the molecular ion at m/z 496. The other ions appeared at m/z 496.72 [M-CH3], 496 (BASE PEAK), 497, 367, 271, 259, 245, 231, 206, 191, 163, 149, 123, 121, 109, 95, 81, 55. IR spectrum of the compound showed peaks at 1620 cm^{-1} (-C=N-O) and 890 cm^{-1}, 1720 cm^{-1} (-COOMe) for heterocyclic ring. It showed UV absorption maximum at 223 nm (ε = 5169) for disubstituted furazine derivative. The ^{1}HNMR were 0.77 (d, 3H, J = 7Hz), 0.78, 0.86 (d, 3H, J = 7Hz), 0.97, 1.10. It showed the presence of six tertiary methyls. A pair of one-proton doublets each at 2.1 (J = 16 Hz) ppm and 3.15 (J = 16 Hz) ppm may be due to the germinal coupling of the C-1 proton which were adjacent to the furazine ring. Thus from spectral analysis the structure of the compound 14 has been established as 28-carbomethoxy-lupan- [2,3-C]- 1′,2′,5′-oxadiazole (compound J).



(7a)

Dioxim of methyl dihydrobetulinate



(7b)

28-Carbomethoxy lupan [2,3-C]-1′,2′,5′-oxadiazole

Antifungal and antibacterial activity of the compound-F was also tested following disc diffusion method. Results of the test have been presented in the table-3.6, table-3.7 & plate-7. Compound-F did not show any antifungal activity against *C. eragrostidis* but it could check growth of the two fungi (*C.gloeosporioides* and *F.equiseti*) as evident from the results presented in table-3.6. The diameter of inhibition zones ranged between 1.2 to 2.5cms. The results of antibacterial activity of Compound-F have been presented in table-3.7. From the results it was evident that growth of *R. solanacearum* could be inhibited at 200ppm concentration and above. Growth of all other bacteria (*Xanthomonas sp, P.syringae* and *E.carotovora*) tested was controlled even at concentration of 100 ppm. Largest diameter (2.8cms) of antifungal zone was observed at 500ppm concentration of the compound against *R. solanacearum*.

Table 3.6: Antifungal Activity of 2,2-dibromo methyldihydrobetulonate (compound'F')

Fungal organism	Concentrations of compounds(ppm)	Diameter of inhibition zone(cm)*
Colletotrichum gloeosporioides	100	1.2
	200	1.5
	300	1.9
	400	2.2
	500	2.5
Fusarium equiseti	100	1.6
	200	1.8
	300	1.9
	400	2.0
	500	2.3
Curvularia eragrostidis	100	-
	200	-
	300	-
	400	-
	500	-

*mean of three replications; - indicates no inhibition zone formed

Section 2: Antimicrobial activity

2.1: Materials and Methods:

Details of the materials and methods have already been mentioned in chapter II section 2.1

2.2. Results:

Compound F, chemical name 2,2-dibromo methyl dihydrobetulonate was extracted from the bark of *Bischofia javanica*, belongs to the family Euphorbiaceae [details of extraction procedure was given in chapter-III, section 1.1]. Pharmacological research [5] on the bark of the plant suggested that the bark has usable antifungal and antimicrobial properties [28-30,33,34].

From the results presented in table-3.5 it was found that 2,2-dibromo methyldihydrobetulonate (Compound-F) was highly effective in controlling the spore germination of *Colletotrichum gloeosporioides, Fusarium equiseti* and *Curvularia eragrostidis*. Spore germination of all the fungal pathogens was reduced to a significant level by all the five concentrations (100,200,300,400 & 500ppm) of the compound tested.

Table3.5: Percent inhibition of spore germination of *Colletotrichum gloeosporioides, Fusarium equiseti* and *Curvularia eragrostidis* by 2,2-dibromo methyldihydrobetulonate (Compound-F) (when control raised to 100).

Fungal organism	Concentrations of compound(ppm)	Range of germtube length (micrometer)	percent germination	Percent Inhibition*
Colletotrichum gloeosporioides	100	12-52	13	87±2.00
	200	08-44	02	98±1.88
	300	08-32	01	99±1.69
	400	-	-	100±1.00
	500	-	-	100±1.00
Fusarium equiseti	100	08-16	25	75±1.50
	200	04-12	15	85±1.75
	300	04-12	07	93±1.52
	400	04-08	04	96±1.88
	500	04-08	nil	100±1.00
Curvularia eragrostidis	100	40-112	23	77±1.20
	200	32-72	12	88(S=±1.34)
	300	28-56	10	90(S=±1.99)
	400	20-48	08	92(S=±1.58)
	500	16-32	06	94(S=±1.35)

*Data after ± indicates standard error value

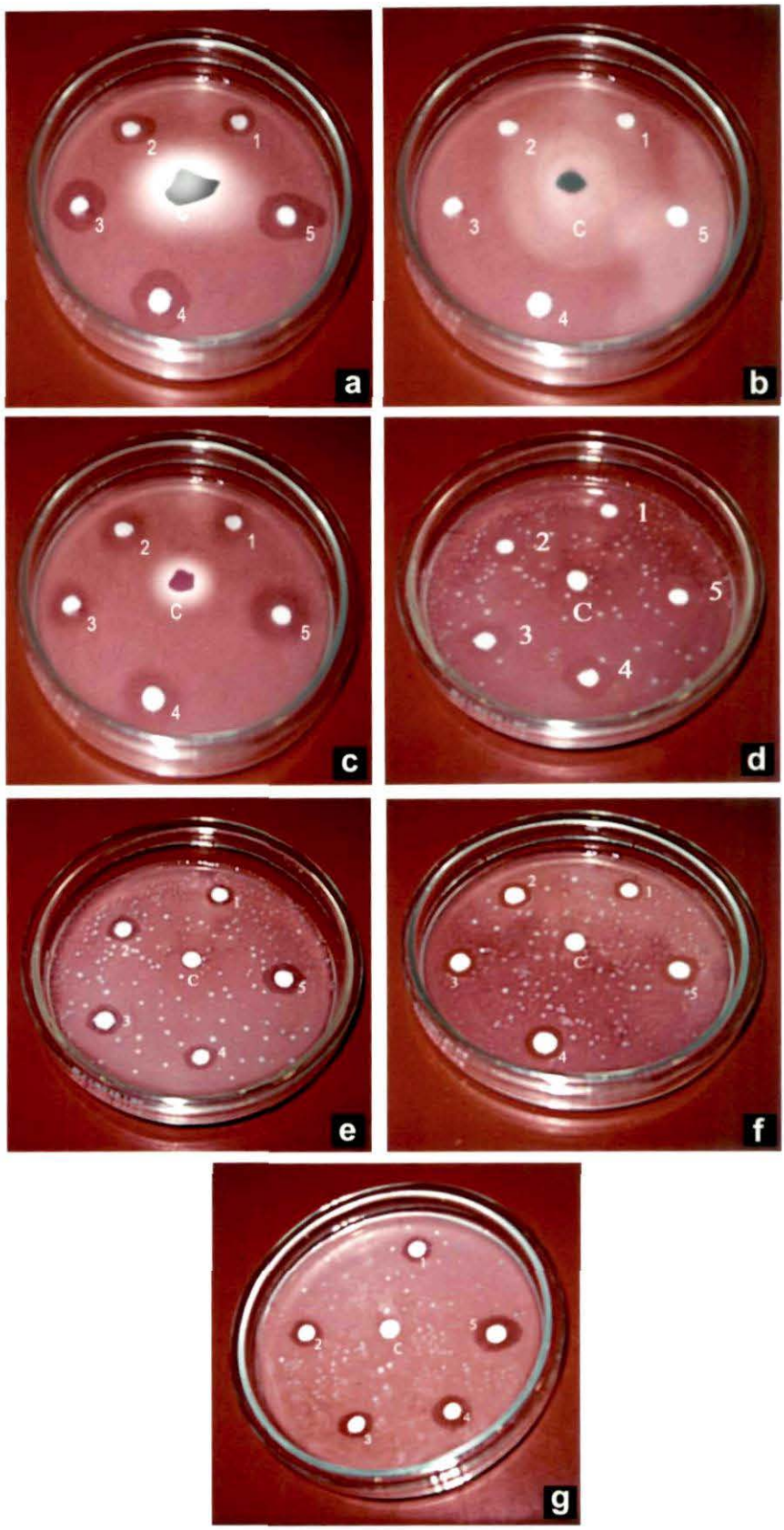


Plate 6: Disc diffusion test for anti microbial activity of compound F:(2,2-dibromomethyl dihydrobetulonate); at five different concentrations* against (a) *Colletotrichum gloeosporioides* (b) *Curvularia eragrostidis* (c) *Fusarium equiseti* (d) *Ralstonia solanacearum* (e) *Pseudomonas syringae* (f) *Erwinia carotovora*. (g) *Xanthomonas* sp.

* 1=100ppm, 2=200 ppm, 3=300 ppm, 4=400 ppm, 5=500ppm, c=DMSO control.

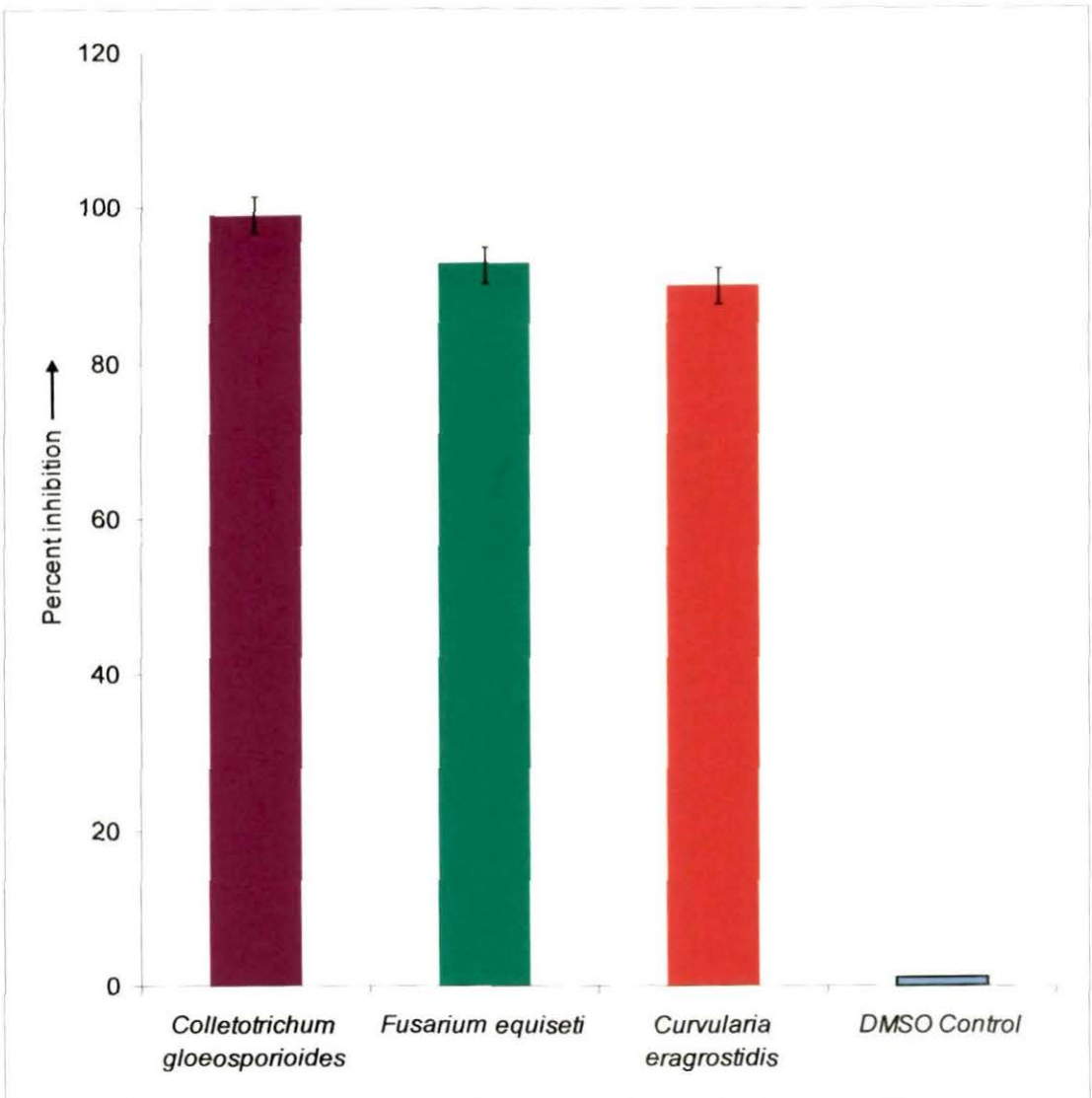


Fig. 33: Percent inhibition of spore germination at 300 ppm concentration of compound F (2,2-dibromo methyldihydrobetulonate).

Table 3.7: Antibacterial activity of 2,2dibromomethyldihydrobetulonate (Compound'F')

Bacterial organism	Concentrations of compound (ppm)	Diameter of inhibition zone(cm)*
Ralstonia solanacearum	Control	-
	100	0.4
	200	2.4
	300	2.5
	400	2.7
	500	2.8
Xanthomonas sp	control	-
	100	1.5
	200	1.7
	300	1.9
	400	2.1
	500	2.2
Pseudomonas syringae	Control	-
	100	1.0
	200	1.2
	300	1.5
	400	1.6
	500	1.7
Erwinia carotovora	Control	-
	100	1.1
	200	1.4
	300	1.7
	400	1.9
	500	2.2

*mean of three replications; - indicates no inhibition zone formed

In the present study, 2α-Bromo methyldihydrobetulonate (Compound-G) was used as seventh compound for control of fungal and bacterial pathogens. Initially, the spore germination bioassay was performed. The results of the spore germination bioassay have been presented in Table 3.8. More than 90% reduction in spore germination was experienced at 500ppm concentration of the compound-G against the three fungi (*Colletotrichum gloeosporioides, Fusarium equiseti* and *Curvularia eragrostidis*). Cent percent spores of *Fusarium equseti* could not germinate in presence of the compound G even at 100 ppm concentration.

Table 3.8: Percent inhibition of spore germination of *Colletotrichum gloeosporioides*, *Fusarium equiseti* and *Curvularia eragrostidis* by 2α-Bromomethyldihydrobetulonate (Compound 'G') (when control raised to 100)

Fungal organism	Concentrations of compound(ppm)	Range of germ tube Length(micrometer)	percent germination	Percent Inhibition*
Colletotrichum gloeosporioides	100	12-52	26	74±1.30
	200	08-40	11	89±1.94
	300	08-32	10	90±1.66
	400	04-32	07	93±1.78
	500	04-20	04	96±1.90
Fusarium equiseti	100	08-20	12	88±1.80
	200	08-16	07	93±1.60
	300	04-12	04	96±1.68
	400	04-12	01	99±1.00
	500	-	-	100±1.00
Curvularia eragrostidis	100	40-108	21	79±1.40
	200	32-80	17	83±1.95
	300	28-56	13	87±1.84
	400	24-48	08	92±1.36
	500	20-40	07	93±2.00

*Data after ± indicates standard error value

Results presented in table-3.9 and plate-8 represents the antifungal activity of Compound-G (through disc diffusion bioassay) at five different concentrations. From the results of antifungal activity of compound–G (2α-Bromo methyldihydrobetulonate), it was evident that the compound at all the five different concentrations could check the growth of two pathogens (*C.gloeosporioides* and *F.equiseti*) but it could not control spore germination of *C. eragrostidis*.

In table 3.10, the results of antibacterial activity of compound-G (2α-bromomethyldihydrobetulonate) have been presented. Compound-G was effective from 100ppm concentration against the bacteria tested. Diameter of inhibition zones ranged between 0.7 to 2.1 cm. Best control (diameter of inhibition zone 2.1cm) of growth of the bacterium (*P.syringae*) was observed at 500ppm concentration.

Table 3.9: Antifungal activity of 2α-Bromo methyldihydrobetulonate (Compound 'G')

Fungal organism	Concentrations ofcompounds(ppm)	Diameter of inhibition zone(cm)*
Colletotrichum gloeosporioides	100	0.8
	200	0.9
	300	1.0
	400	1.2
	500	1.4
Fusarium equiseti	100	1.4
	200	1.7
	300	1.9
	400	2.0
	500	2.1
Curvularia eragrostidis	100	-
	200	-
	300	-
	400	-
	500	-

Table3.10: Antibacterial activity of 2α-bromomethyldihydrobetulonate (compound 'G')

Bacterial organism	Concentrations of compound (ppm)	Diameter of inhibition zone(cm)*
Ralstonia solanacearum	Control	-
	100	0.7
	200	1.0
	300	1.2
	400	1.4
	500	1.8
Xanthomonas sp	control	-
	100	0.5
	200	0.8
	300	1.0
	400	1.3
	500	1.5
Pseudomonas syringae	Control	-
	100	1.0
	200	1.2
	300	1.5
	400	1.8
	500	2.1
Erwinia carotovora	Control	-
	100	0.7
	200	0.8
	300	1.0
	400	1.3
	500	1.8

*mean of three replications; - indicates no inhibition zone formed

Compound-H (28-carbomethoxylup-1(2)-en-3-one) was the eight compound tested for the antimicrobial activity against three fungal and four bacterial pathogens. Details of the extraction and synthesis of the compound have been mentioned in the section- 1of chapter-III. From the results presented in table 3.11and plate-9 it was found that 100ppm concentration of the compound could reduce spore germination up to 73%, 77% and 87% respectively in case of *Fusarium equiseti, Colletotrichum gloeosporioides* and *Curvularia eragrostidis.* More than 95% inhibition of spore germination was experienced when 500 ppm concentration of the compound was used against the three test pathogens.

Table 3.11: Percent inhibition of spore germination of *Colletotrichum gloeosporioides, Fusarium equiseti* and *Curvularia eragrostidis* by 28-carbomethoxylup-1(2)-en-3-one (Compound 'H') (when control raised to 100)

Fungal organism	Concentrations of compound (ppm)	Range of germ tube length(micrometer)	percent germination	Percent Inhibition*
Colletotrichum gloeosporioides	100	12-44	23	77±1.13
	200	12-40	05	95±1.80
	300	08-36	03	97±1.65
	400	08-24	02	98±1.88
	500	04-20	01	99±1.66
Fusarium equiseti	100	12-20	27	73±1.70
	200	08-20	12	88±1.38
	300	08-20	10	90±1.55
	400	04-12	08	92±1.30
	500	04-08	05	95±1.45
Curvularia eragrostidis	100	36-100	13	87±1.87
	200	24-72	08	92±1.99
	300	28-56	07	93±1.32
	400	24-48	05	95±1.70
	500	20-40	04	96±1.80

*Data after ± indicates standard error value

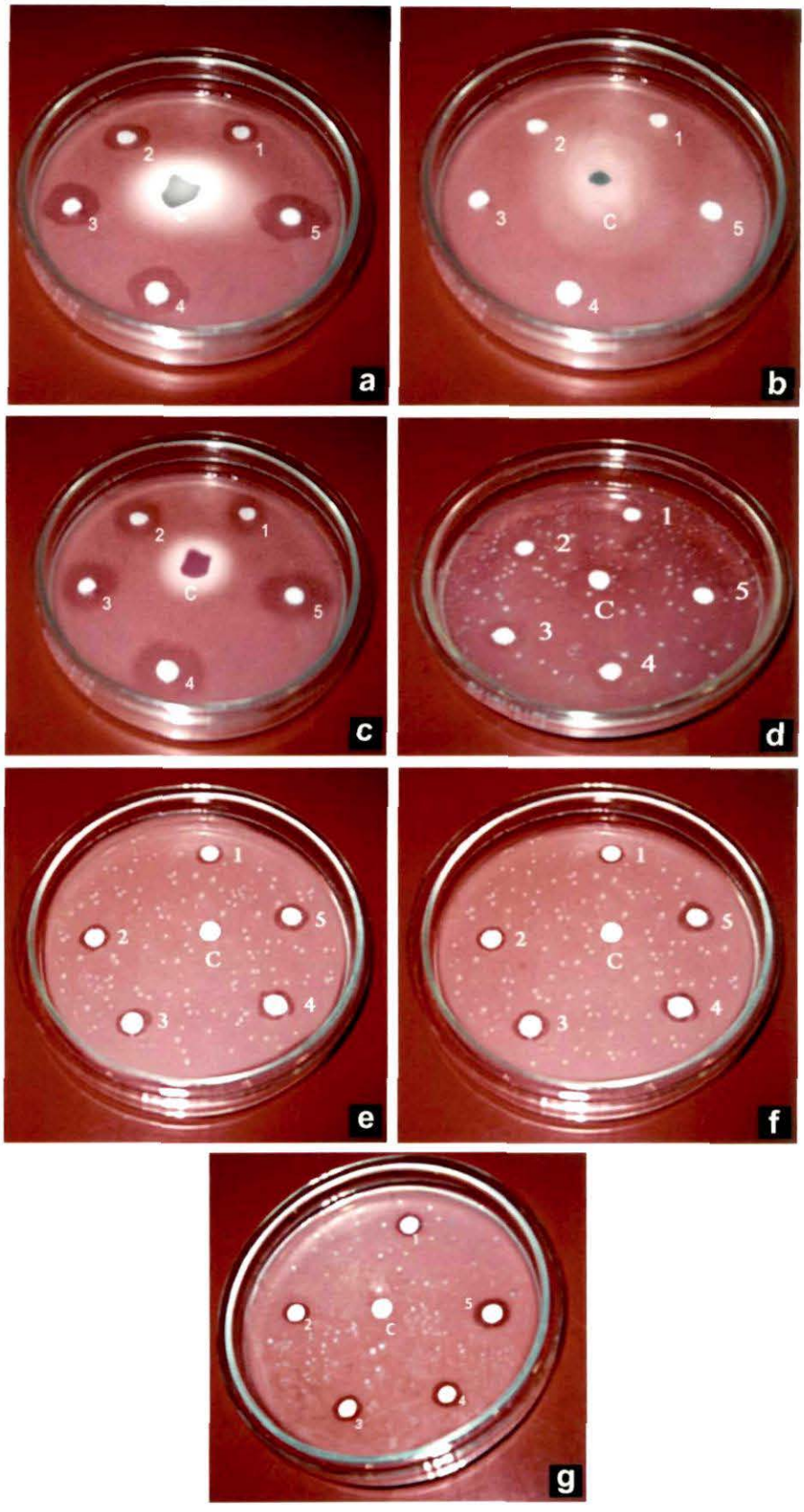


Plate 7: Disc diffusion test for anti microbial activity of compound G: (2α-bromo methyldihydrobetulonate); at five different concentrations* against (a) *Colletotrichum gloeosporioides* (b) *Curvularia eragrostidis* (c) *Fusarium equiseti* (d) *Ralstonia solanacearum* (e) *Pseudomonas syringae* (f) *Erwinia carotovora*. (g) *Xanthomonas* sp.

* 1=100ppm, 2=200 ppm, 3=300 ppm, 4=400 ppm, 5=500ppm, c=DMSO control.

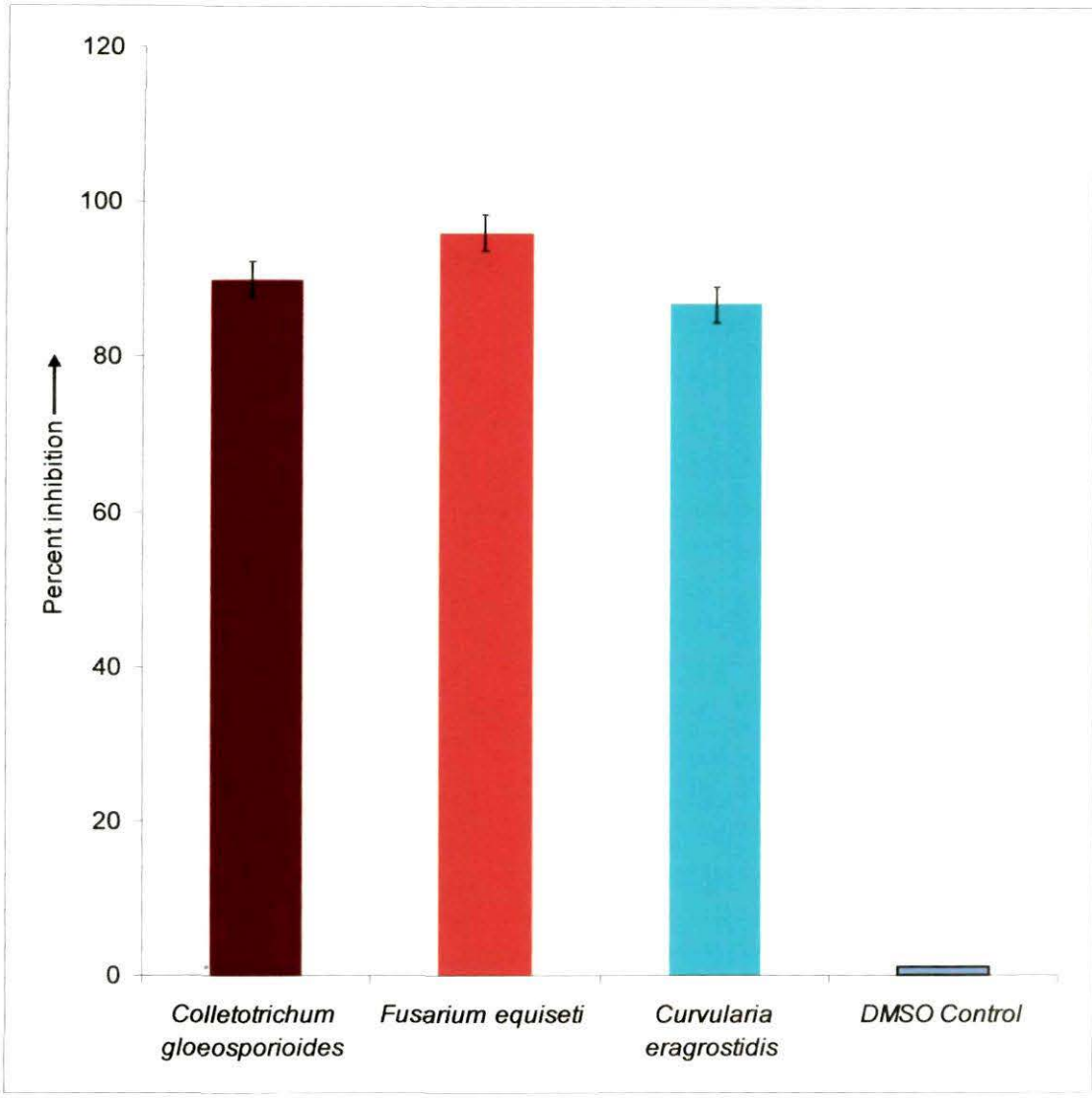


Fig. 34: Percent inhibition of spore germination at 300 ppm concentration of compound G (2α-bromomethyldihydrobetulonate).

Results of the disc diffusion method for evaluation of antimicrobial activity of Compound-H have been presented in table 3.12, 3.13 & plate-9. All the five concentrations of compound-H showed antifungal activity against *Colletotrichum gloeosporioides* and *Fusarium equiseti* but it could not inhibit the growth of *Curvularia eragrostidis* even at 500 ppm concentration. Thus the compound was found to be selectively antifungal.

In table 3.13 the results of antibacterial activity of 28-carbomethoxy lup-1(2)-en-3-one (compound H) was presented. Compound-H was effective in all the five concentrations tested. Largest antibacterial zone was found in case of *Pseudomonas syringae* when tested against 500ppm concentration of the compound. Growth of *R.solanacearum, Xanthomonas* sp and *E.carotovora* were also controlled by the compound. Best control was experienced at 500 ppm concentration of the compound.

Table 3.12: Antifungal activity of 28-carbomethoxylup-1(2)-en-3-one (Compound 'H')

Fungal organism	Concentrations of compound (ppm)	Diameter of inhibition zone(cm)*
Colletotrichum gloeosporioides	100	0.6
	200	0.8
	300	1.0
	400	1.2
	500	1.4
Fusarium equiseti	100	1.3
	200	1.5
	300	1.6
	400	1.7
	500	1.9
Curvularia eragrostidis	100	-
	200	-
	300	-
	400	-
	500	-

*mean of three replications; - indicates no inhibition zone formed

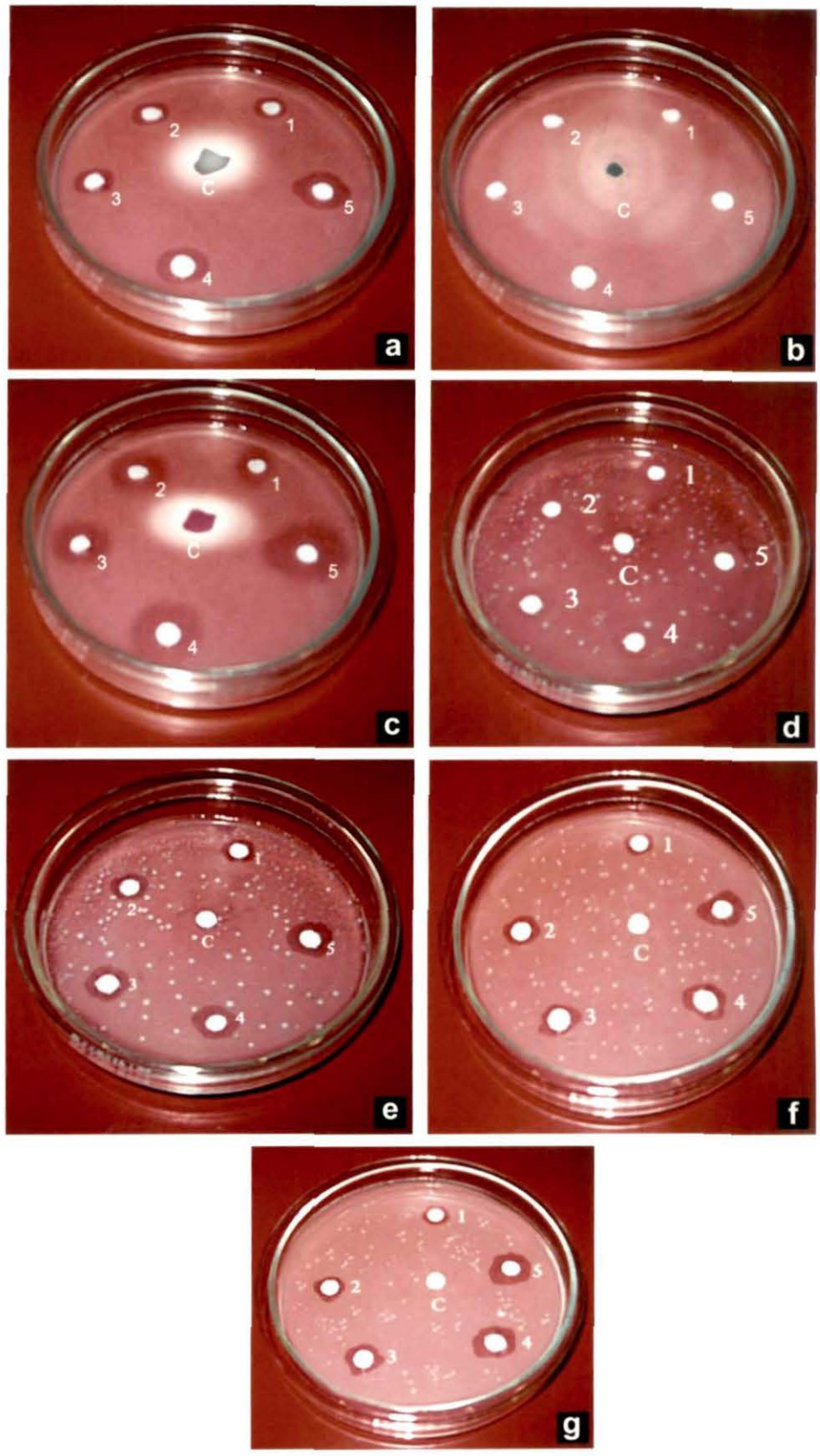


Plate 8: Disc diffusion test for anti microbial activity of compound H: (28-carbomethoxy lup-1(2)-en-3-one); at five different concentrations* against (a) *Colletotrichum gloeosporioides* (b) *Curvularia eragrostidis* (c) *Fusarium equiseti* (d) *Ralstonia solanacearum* (e) *Pseudomonas syringae* (f) *Erwinia carotovora* (g) *Xanthomonas* sp.

* 1=100ppm, 2=200 ppm, 3=300 ppm, 4=400 ppm, 5=500ppm, c=DMSO control.

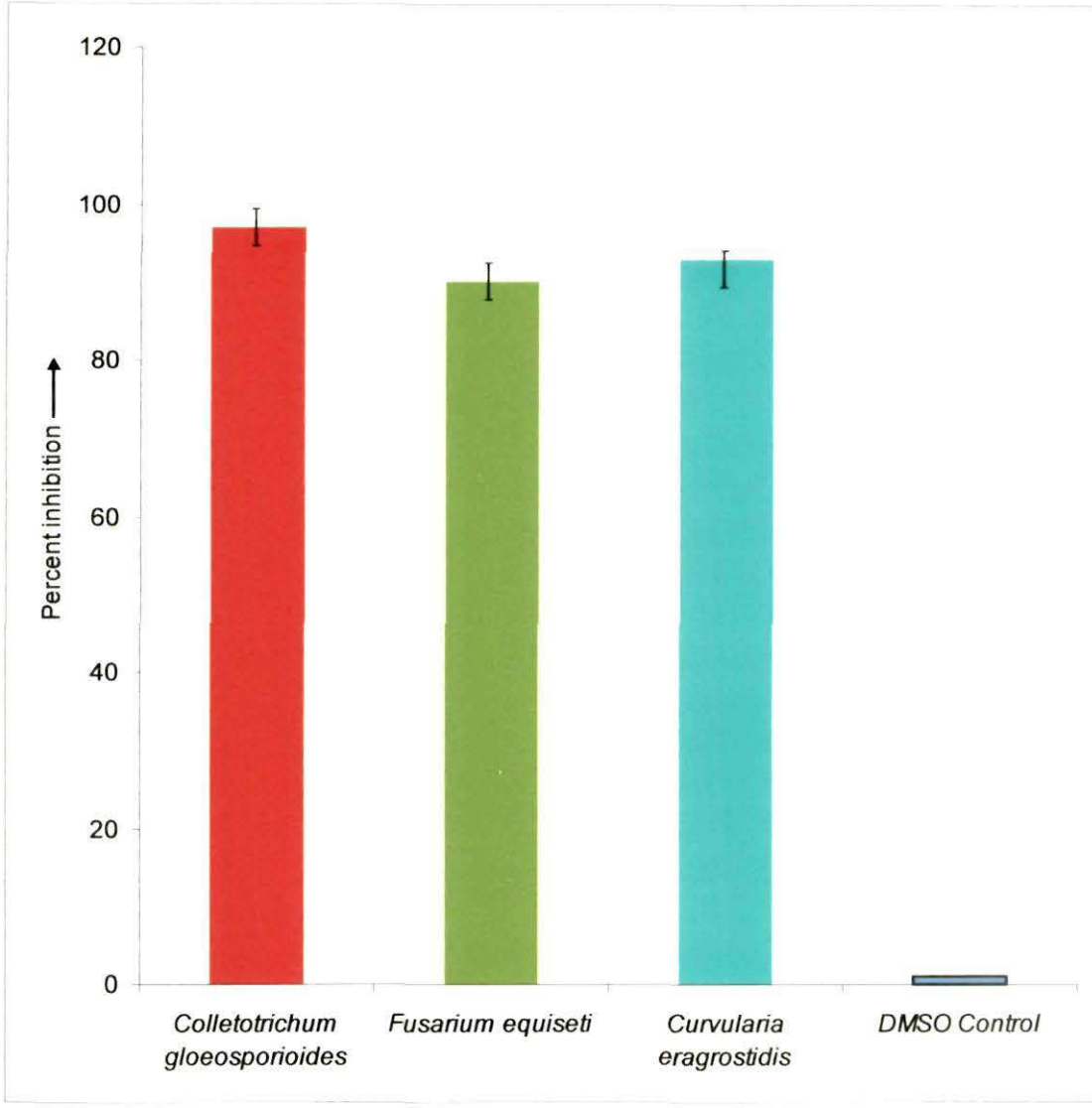


Fig. 35: Percent inhibition of spore germination at 300 ppm concentration of compound H (28-carbomethoxy lup-1(2)-en-3-one).

Table 3.13: Antibacterial activity of 28-carbomethoxy lup-1(2)-en-3-one (compound-H)

Bacterial organism	Concentrations of compound (ppm)	Diameter of inhibition zone(cm)*
Ralstonia solanacearum	Control	-
	100	0.7
	200	1.0
	300	1.2
	400	1.4
	500	1.8
Xanthomonas sp	control	-
	100	0.5
	200	0.8
	300	1.0
	400	1.3
	500	1.5
Pseudomonas syringae	Control	-
	100	1.0
	200	1.2
	300	1.5
	400	1.8
	500	2.1
Erwinia carotovora	Control	-
	100	0.7
	200	0.8
	300	1.0
	400	1.3
	500	1.8

*mean of three replications; - indicates no inhibition zone formed

Compound-I (identical as 28-Carbomethoxy lupan[2,3-C]-1́,2́,5́-oxadiozole) was formed by cyclisation of 28-carbomethoxy-2,3-dioximinolupane. Details of the formation of the compound was mentioned in the section-1of chapter III. From the results presented in the table 3.14, it was found that the antifungal activity of 28-carbomethoxy lupan [2,3-C]-1́,2́,5́-oxadiozole (compound-I) was effective at higher concentrations. More than 90% inhibition of spore germination (of all the three fungi tested) was observed in case of 400ppm concentration of the compound.

Table3.14: Percent inhibition of spore germination of *Colletotrichum gloeosporioides, Fusarium equiseti* and *Curvularia eragrostidis* by 28-carbomethoxy lupane-[2,3-C]-1',2',5'oxadiozole(compound 'I') (when control raised to 100)

Fungal organism	Concentrations of compound (ppm)	Range of germ tube length (micrometer)	percent germination	Percent Inhibition*
Colletotrichum gloeosporioides	100	12-44	85	15 ±1.18
	200	12-40	32	68±1.50
	300	12-36	21	79±1.70
	400	08-32	05	95±1.85
	500	04-24	04	96±1.92
Fusarium equiseti	100	12-24	84	16±1.45
	200	10-20	18	82±1.60
	300	10-20	17	83±1.41
	400	08-16	10	90±1.86
	500	04-12	05	95±1.63
Curvularia eragrostidis	100	40-88	15	85±1.26
	200	32-80	11	89±1.54
	300	28-68	09	91±1.90
	400	24-56	07	93±1.52
	500	20-48	05	95±1.22

*Data after ± indicates standard error value

In disc diffusion test (presented in the table 3.15 & plate-10) compound–I could not check the mycelia growth of *Curvularia eragrostidis.* But, the other two fungi showed significant antifungal zone of inhibition against the compound. Diameter of inhibition zones was 2.1 and 2.2 cm in case of *Colletotrichum gloeosporioides* and *Fusarium equiseti* respectively at 500 ppm concentration of the compound-I.

In similar experiments (table 3.16 & plate-10) antibacterial activity was also evaluated against the compound. Compound-I was effective against all the four bacteria (*R.solanacerum, Xanthomonas sp, Pseudomonas syringae, E.carotovora*). The diameter of inhibition zones were between 0.6 to 1.5cm. Comparing the diameter of inhibition zones produced by other compounds against the bacteria, compound-I was not a strong antibacterial compound.

Table 3.15: Antifungal activity of 28-carbomethoxylupane[2,3-C]-1′,2′,5′-oxadiozole (compound 'I')

Fungal organism	Concentrations of compounds(ppm)	Diameter of inhibition zone(cm)*
Colletotrichum gloeosporioides	100	1.5
	200	1.6
	300	1.6
	400	1.9
	500	2.1
Fusarium equiseti	100	1.3
	200	1.7
	300	2.0
	400	2.0
	500	2.2
Curvularia eragrostidis	100	-
	200	-
	300	-
	400	-
	500	-

*mean of three replications; - indicates no inhibition zone formed

Table 3.16: Antibacterial activity of 28-carbomethoxy lupane[2,3-C]-1′,2′,5′-oxadiozole (compound-I)

Bacterial organism	Concentrations of compound (ppm)	Diameter of inhibition zone(cm)*
Ralstonia solanacearum	Control	-
	100	0.6
	200	0.8
	300	0.9
	400	1.0
	500	1.2
Xanthomonas sp	control	-
	100	0.4
	200	0.7
	300	1.1
	400	1.3
	500	1.5
Pseudomonas syringae	Control	-
	100	0.5
	200	0.6
	300	0.8
	400	0.9
	500	1.1
Erwinia carotovora	Control	-
	100	0.7
	200	0.9
	300	1.0
	400	1.1
	500	1.3

*mean of three replications; - indicates no inhibition zone formed

Phytotoxicity tests of Betulinic Acid and its derivatives:

Phytotoxicity of the four compounds were tested in tomato plants (of Priya variety popularly cultivated in the present study area). To test phytotoxicity, tomato plants were grown in earthen pots [size 15cm (diameter) and 15 cm (height)]. The pots were maintained in the experimental garden of the Department of Botany with normal watering. Fifty such pots containing plants (each 10 cm in height) were kept in a net house. The plants were grouped into five sets and each set contained 10 plants each. Four compounds (at 100 ppm concentration) were sprayed in six sets separately and the fifth set was sprayed with sterile distilled water. The plants were observed up to seven days. No phytotoxicity was observed at 100ppm concentration of the compounds when compared with the control set which was sprayed with the sterile distilled water (table-3.17).

Table 3.17: Phytotoxicity of betulinic acid and its derivatives

Compounds	Concentrations (ppm)	Morphological & Physiological abnormalities abnormalities			
		1day	3day	5day	7day
F	100	Plants alive, no significant changes Root-germinated Shoot- germinated	Plants alive, no significant changes Root-germinated Shoot- germinated	Plants alive, no significant changes Root-germinated Shoot- germinated	Plants alive, no significant changes Root-germinated Shoot- germinated
G	100	Plants alive, no significant changes Root-germinated Shoot- germinated	Plants alive, no significant changes Root-germinated Shoot- germinated	Plants alive, no significant changes Root-germinated Shoot- germinated	Plants alive, no significant changes Root-germinated Shoot- germinated
H	100	Plants alive, no significant changes Root-germinated Shoot- germinated	Plants alive, no significant changes Root-germinated Shoot- germinated	Plants alive, no significant changes Root-germinated Shoot- germinated	Plants alive, no significant changes Root-germinated Shoot- germinated
I	100	Plants alive, no significant changes Root-germinated Shoot- germinated	Plants alive, no significant changes Root-germinated Shoot- germinated	Plants alive, no significant changes Root-germinated Shoot- germinated	Plants alive, no significant changes Root-germinated Shoot- germinated
Control	Sterile distilled water	Plants alive, no significant changes Root-germinated Shoot- germinated	Plants alive, no significant changes Root-germinated Shoot- germinated	Plants alive, no significant changes Root-germinated Shoot- germinated	Plants alive, no significant changes Root-germinated Shoot- germinated

2.3. DISCUSSION

Chapter III deals with an evergreen woody tree *Bischofia javanica* belongs to the family Euphorbiaceae. Betulinic acid was obtained through phytochemical extraction of *Bischofia javanica*. Then Methyl betulinate was isolated through estarification of betulinic acid and characterized. After that Methyl dihydrobetulinate was prepared by hydrogenation of methylbetulinate and was also characterized. Methyldihydrobetulonate was prepared by Jone's oxidation of betulinic acid and characterized. Treatment of methyldihydrobetulonate with N-bromosuccinimide, 2,2-dibromomethyldihydro-betulonate (compound F) and 2α-bromomethyldihydrobetulonate (compound G) were formed and characterization of these compounds were done. 28-carbomethoxy-lup (2)-en-3-one, compound H was prepared by dehydrobromination of 2α-bromodihydromethylbetulonate and characterized. Another derivative of betulinic acid named 28-carbomethoxy Lupane-[2,3-C]-1′,2′,5′- oxadiozole, called compound-I was prepared by cyclisation of 28-carbomethoxy 2,3-dioximolupane in dry DMF under microwave irradiation (100w,100^{0}C) for 10 min.

Compound-F (2,2-dibromo methyldihydrobetulonate) significantly controlled (100% inhibition) spore germination of *Colletotrichum gloeosporioides* and *Fusarium equiseti* at highest concentration (500ppm) tested. *Curvularia eragrostidis* was also controlled (inhibition was 94% at 500ppm concentration). From the results of disc diffusion test, it was evident that vegetative growth of *Curvularia eragrostidis* could not be controlled by compound-F. But it could control the vegetative growth of *Colletotrichum gloeosporioides* and *Fusarium equiseti* significantly (showed 2.5cm and 2.3cm diameter of inhibition zones respectively). Three bacteria (*Xanthomonas sp, Pseudomonas syringae* and *Erwinia carotovora*), tested against compound-F showed inhibition of growth. *R. solanacearum* could not be controlled at 100ppm concentration but it could be controlled significantly at 200ppm concentration.The inhibition zone diameter was found to be 2.4cm. However, the other three bacteria (*Xanthomonas sp, Pseudomonas syringae* and *Erwinia caratovora*) could be controlled at lowest concentration (100ppm) of the compound.

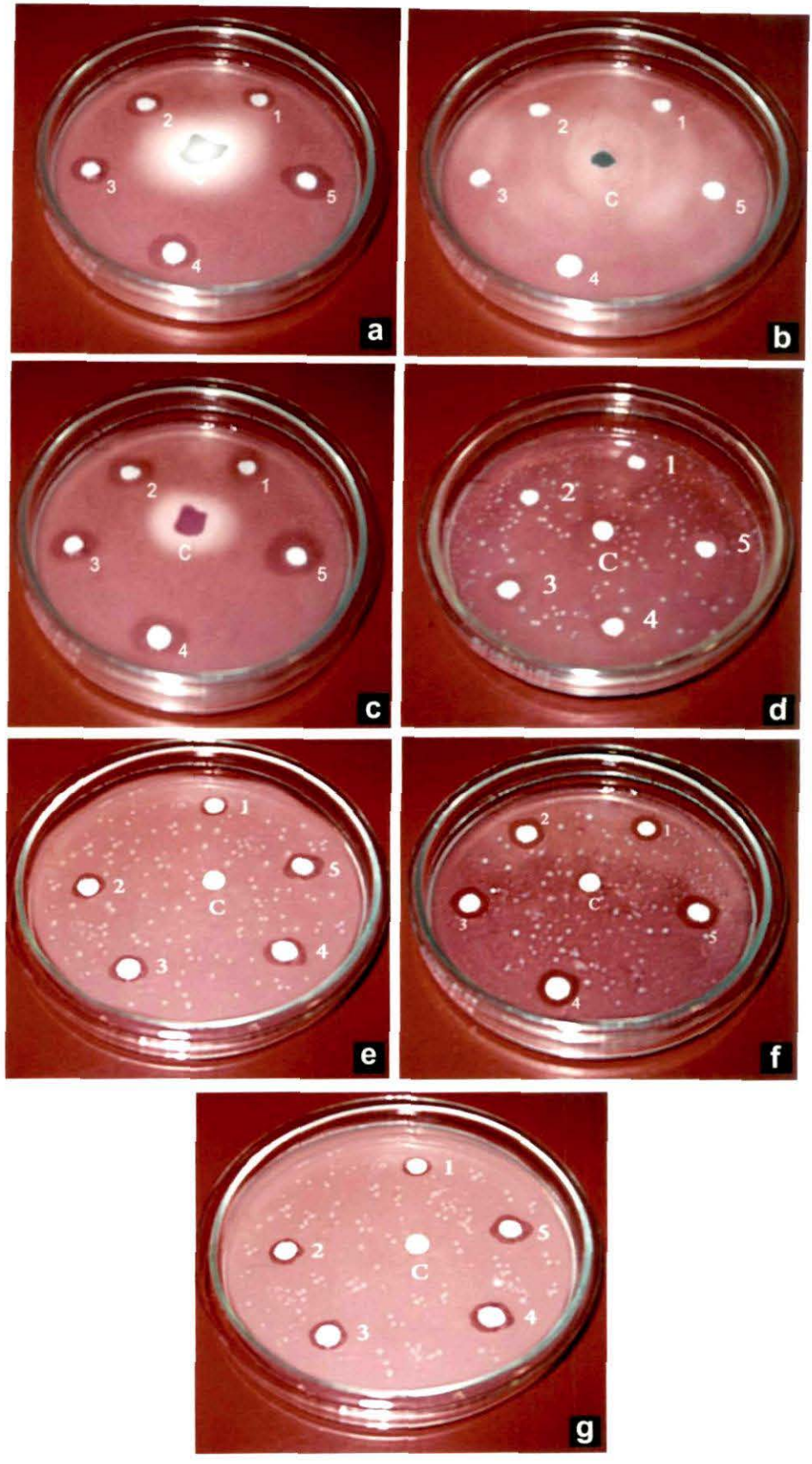


Plate 9: Disc diffusion test for anti microbial activity of compound I:(28 carbomethoxy lupane - [2,3-C]-**1′,2′,5′**-oxadiozole); at five different concentrations* against (a) *Colletotrichum gloeosporioides* (b) *Curvularia eragrostidis* (c) *Fusarium equiseti* (d) *Ralstonia solanacearum* (e) *Pseudomonas syringae* (f) *Erwinia carotovora*. (g) *Xanthomonas* sp.

* 1=100ppm, 2=200 ppm, 3=300 ppm, 4=400 ppm, 5=500ppm, c=DMSO control.

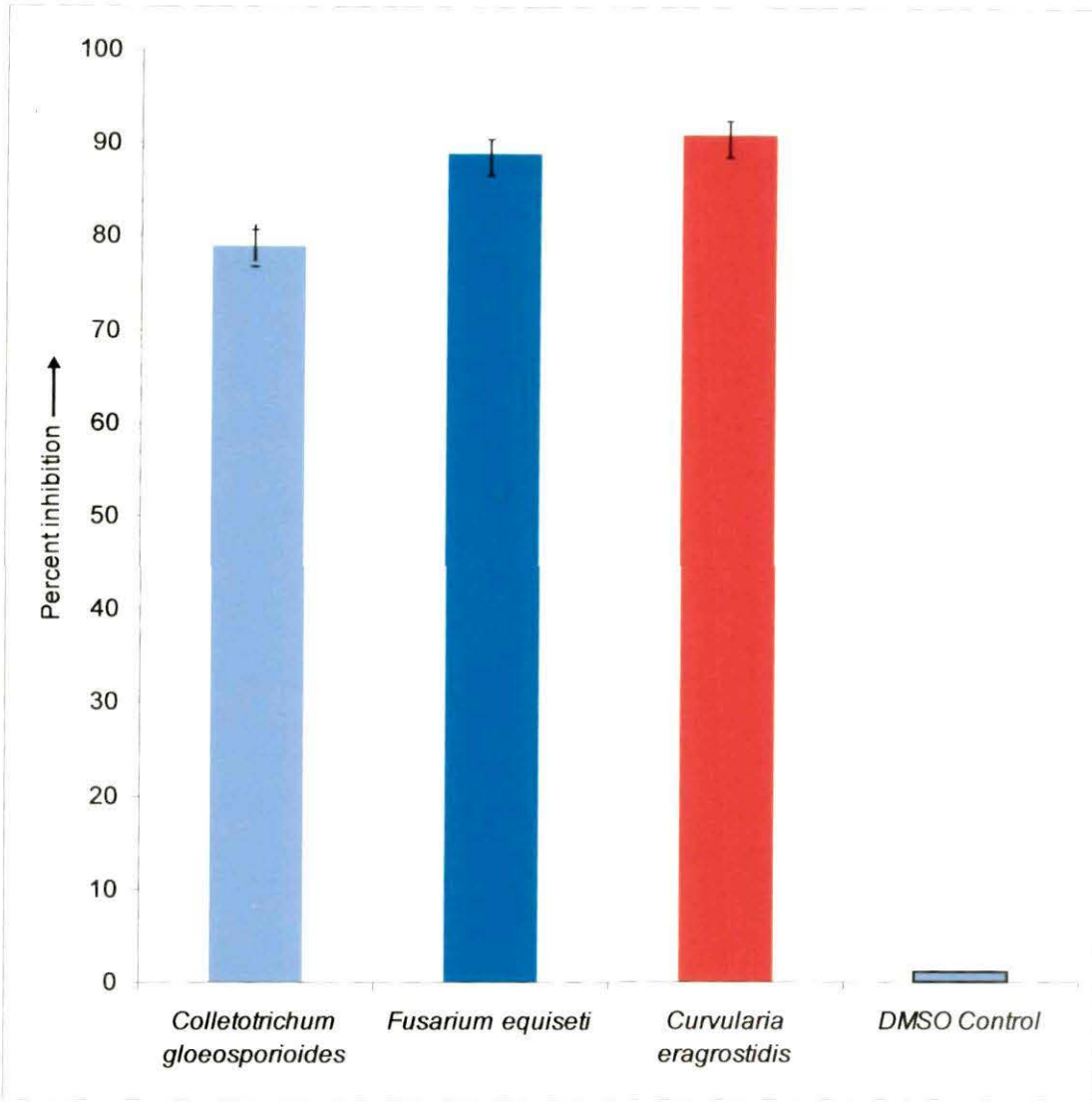


Fig. 36: Percent inhibition of spore germination at 300 ppm concentration of compound I (28-carbomethoxy lupane[2,3-C]-1¢,2¢,5¢-oxadiazole).

From the results presented in table-3.8 it was evident that compound-G (2α-Bromo methyldihydrobetulonate) could inhibit more than 90% of spore germination at higher concentrations (400ppm & 500ppm) tested. More than 70% inhibition was also recorded at the lowest concentration (100ppm) tested. In disc diffusion test Compound-G could check *Colletotrichum gloeosporioides* and *Fusarium equiseti* but *Curvularia eragrostidis* was not controlled even at highest concentration (500ppm) tested. Compound-G controlled the growth of all the four bacteria (*Ralstonia solanacearum, Xanthomonas sp, Pseudomonas syringae* and *Erwinia carotovora*) at the five concentrations tested. But significant inhibition zone (more than 1.0 cm in diameter) was found at 300ppm concentration and above. In case of *Erwinia carotovora* and *Xanthomonas sp* more than 1.0 cm diameter of inhibition zones were observed at 200ppm concentration. But *Ralstonia solanacearum* and *Pseudomonas syringae* could be controlled significantly at all the five concentrations tested. Diameter of inhibition zones were more than1.0cm.

Compound H (28-carbomethoxylup-1(2)-en-3-one) reduced germination of spores of all the three fungi (*Colletotrichum gloeosporioides, Fusarium equiseti* and *Curvularia eragrostidis*) even at lowest concentration(100ppm). But more than 95% germination was checked at 500ppm concentration. In disc diffusion test growth of *Colletotrichum gloeosporioides* and *Fusarium equiseti* was controlled by the compound-H but the growth of *Curvularia eragrostidis* could not be controlled even at 500ppm concentration. The compound showed selective antifungal activity.

Compound-H showed best antibacterial activity against *Pseudomonas syringae.* At 100ppm concentration of the compound diameter of inhibition zone was 1.0cm. The other three bacteria showed moderate antibacterial activity. *Xanthomonas sp* and *R. solanacearum* showed low diameter of inhibition zone (0.5cm and 0.7cm respectively).

Unlike the other three compounds (F,G,H), compound-I (28 Carbomethoxy lupan[2,3-C]-1',2',5'-oxadiozole) was less effective in controlling germination of spores. *But Curvularia eragrostidis* was controlled by the compound significantly. About 85% of spores did not germinate at 100 ppm concentration of the compound-I. Whereas nearly 16% spores of *Colletotrichum gloeosporioides* and *Fusarium equiseti* could not

germinate at that concentration. Thus compound-I showed more effectiveness towards the inhibition of spore germination of *Curvularia eragrostidis* than *Colletotrichum gloeosporioides, Fusarium equiseti.* In contrast to the germination of spores, when growth of *Curvularia eragrostidis* was tested against five different concentrations of compound-I, no inhibition zone was found. This indicated that although spore germination of *Curvularia eragrostidis* could be controlled but mycelia growth was not controlled by the compound. The other two fungi showed significant zone of inhibition in disc diffusion test and thus showed the effectiveness of the compound to control the growth of two fungi mentioned. Compound-I showed antibacterial activity at higher concentrations (300ppm, 400ppm and 500ppm) towards all the four bacteria (*Ralstonia solanacearum, Xanthomonas sp, Pseudomonas syringae,and Erwinia carotovora*). Thus the compound I showed moderate antibacterial activity.

On the basis of phytotoxicity test 100ppm concentration of compound F,G,H, I were not phytotoxic. Hence the compounds which are effective in controlling pat at 100ppm concentration and also not phytotoxic may be recommended for cont the pathogens *in vivo* or in field condition.

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■ CHAPTER - IV

Title: Extraction and characterization of Cerin and Friedelin from Quarcus suber, preparation of its derivatives and antimicrobial activity of each of them

Chapter IV deals with isolation, characterization and preparation of two antimicrobial derivatives from plant *Quarcus suber*. *Q. suber* is a plant commonly called Cork Oak, belongs to the family Fagaceae (Beech family). This particular plant mainly found in siliceous hills on the littoral [1]. This medium sized, evergreen tree is a primary source of cork for wine bottle stopper and other uses such as cork flooring [2]. It is a native of southwest Europe and northwest Africa. It grows up to 20 meters. The leaves are 4 to 7 cm long, lobed or toothed, dark green upper surface and pale lower surface. Sometimes the leaf margins curve downwards.

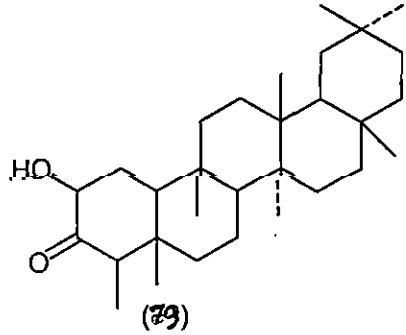
The tree forms a thick, rugged bark, containing high levels of suberin. The cork cambium layer of bark develops considerable thickness and at every 9 to 12 years cork can be harvested [3]. The harvesting of cork does not harm the tree. However, no trees are cut down during harvesting process. Only the bark is extracted, and a new layer of cork is produced, making it a renewable resource.

The plant has medicinal properties. Any gall produced on the tree is strong astringent and can be used in the treatment of Hemorrhages, chronic diarrhea, dysentery etc. The seeds are used as food [4]. It can be dried, ground into a powder and are used as a thickening in stews or mixed with cereals for making bread. It contains bitter tannins too [5]. Cerin and friedelin have been isolated from the plant which has both antibacterial as well as antifungal activity.

Section 1: Extraction and characterization of pentacyclic triterpenoids (Cerin and Friedelin) from *Quarcus suber*

1.1: Isolation of Cerin from bark cork:

3 kg of dried finely powdered cork was extracted with petroleum ether in a soxhlet apparatus for 72 hours. After removal of solvent, a brown gummy residue was separated out. The dried residue was dissolved in minimum volume of hot chloroform and kept overnight in dark. The desired compound Cerin was crystallized out from the solvent then filtered and once again recrystalized from hot chloroform. After that slightly yellow needle shaped crystals of Cerin were separated out from the solvent.

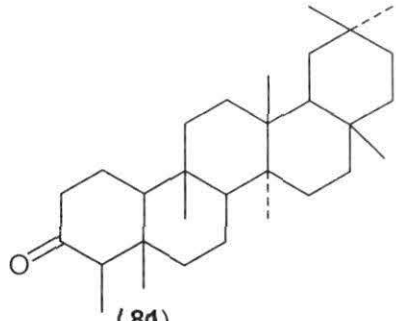


(79)
Cerin

1.2: Isolation of friedelin from bark cork:

3 kg of finely powdered cork was extracted with petroleum ether in a soxhlet apparatus for 18 hours. After removal of the solvent a white solid separated out. The solid was dissolved in minimum volume of benzene and chromatographed over silica gel column, developed with petroleum ether. Elution of the column with petroleum ether gave shining crystals of friedelin, m.p. 248^0-252^0C.

IR: 1720 cm^{-1} (-C=O)



(80)

Friedelin

1.3: General experimental detail:

The melting points were determined by open capillary method. The NMR spectra were recorded in $CDCl_3$ solutions at ambient temperature on a Bruker Avance 300 MHz-FT NMR spectrometer using 5 mm BBO probe. The chemical shift δ was given in ppm related to tetra methylsilane (TMS) as internal standard. The coupling constants (*J*) were reported in Hz.

The IR spectram was recorded in Shimadzu FT-IR spectrophotometer in KBr discs.

1.4: Plant material:

Fresh corks were collected from the local market and were cut into small pieces. These were then used for further study.

1.5: Preparation of plant extract:

The cork material was extracted with pet ether in a soxhlet apparatus for 72 hours. The solvent was recovered that yielded a deep brown gummy residue. This crude extract was dissolved in minimum volume of hot chloroform, crocked up and stored for 24 hours. Being less soluble in cold chloroform, cerin crystallized out at the bottom of the container as slightly yellowish crystals. It was then filtered over a sintered Buckner funnel and washed thrice with cold chloroform. All the washings and the supernatant liquid were then mixed and purified over a column of silica gel.

Section 2: Antimicrobial activity:

2.1 Material and Methods:

Details of materials and methods, maintenance of stock culture, spore germination bioassay technique and disc diffusion method have been mentioned in sections 2, of Chapter II.

2.2: Results

In the present study compound 'cerin', isolated from cork of *Quarcus suber,* was used as tenth compound to control some fungal and bacterial pathogens of plants. Five different concentrations of cerin were used for spore germination bioassay against three different plant pathogens (*Colletotrichum gloeosporioides, Fusarium equiseti* and *Curvularia eragrostidis*). From the results presented in table 4.1 it was evident that cerin was highly antifungal. No spores germinated even at the lowest concentration (100ppm) tested.

Table 4.1: Percent inhibition of spore germination of *Colletotrichum gloeosporioides, Fusarium equiseti* and *Curvularia eragrostidis* by Cerin (when control raised to 100).

Fungal organism	Concentrations of compound (ppm)	Range of germ tube length (micrometer)	percent germination	Percent Inhibition*
Colletotrichum gloeosporioides	100	-	-	100
	200	-	-	100
	300	-	-	100
	400	-	-	100
	500	-	-	100
Fusarium equiseti	100	-	-	100
	200	-	-	100
	300	-	-	100
	400	-	-	100
	500	-	-	100
Curvularia eragrostidis	100	-	-	100
	200	-	-	100
	300	-	-	100
	400	-	-	100
	500	-	-	100

*mean of three replications

In case of disc diffusion test (table 4.2 & plate-10) also cerin showed high antifungal activity. All the five concentrations of the compound could check the growth of the fungi significantly.

Cerin showed poor antibacterial activity as evidenced from the results presented in table 4.3 and plate-10. No antibacterial activity was recorded by the compound cerin in case of *R. solanacearum, Xanthomonas sp.* and *P. syringae.* Only *E. carotovora* was controlled by cerin at concentration of 300ppm and above.

Table 4.2: Antifungal activity of Cerin

Fungal organism	Concentrations of compound (ppm)	Diameter of Inhibition zone(cm)*
Colletotrichum gloeosporioides	100	1.4
	200	1.6
	300	1.9
	400	2.0
	500	2.2
Fusarium equiseti	100	1.6
	200	1.7
	300	1.9
	400	2.1
	500	2.3
Curvularia eragrostidis	100	1.3
	200	1.5
	300	1.8
	400	2.0
	500	2.2

*mean of three replications;

Table 4.3: Antibacterial activity of Cerin

Bacterial organism	Concentrations of compound (ppm)	Diameter of Inhibition zone(cm)*
Ralstonia solanacearum	Control	-
	100	0.4
	200	0.4
	300	0.4
	400	0.4
	500	0.4
Xanthomonas sp	control	-
	100	0.4
	200	0.4
	300	0.4
	400	0.4
	500	0.4
Pseudomonas syringae	Control	-
	100	0.4
	200	0.4
	300	0.4
	400	0.4
	500	0.4
Erwinia carotovora	Control	-
	100	0.4
	200	0.4
	300	0.8
	400	1.0
	500	1.2

*mean of three replications; - indicates no inhibition zone formed

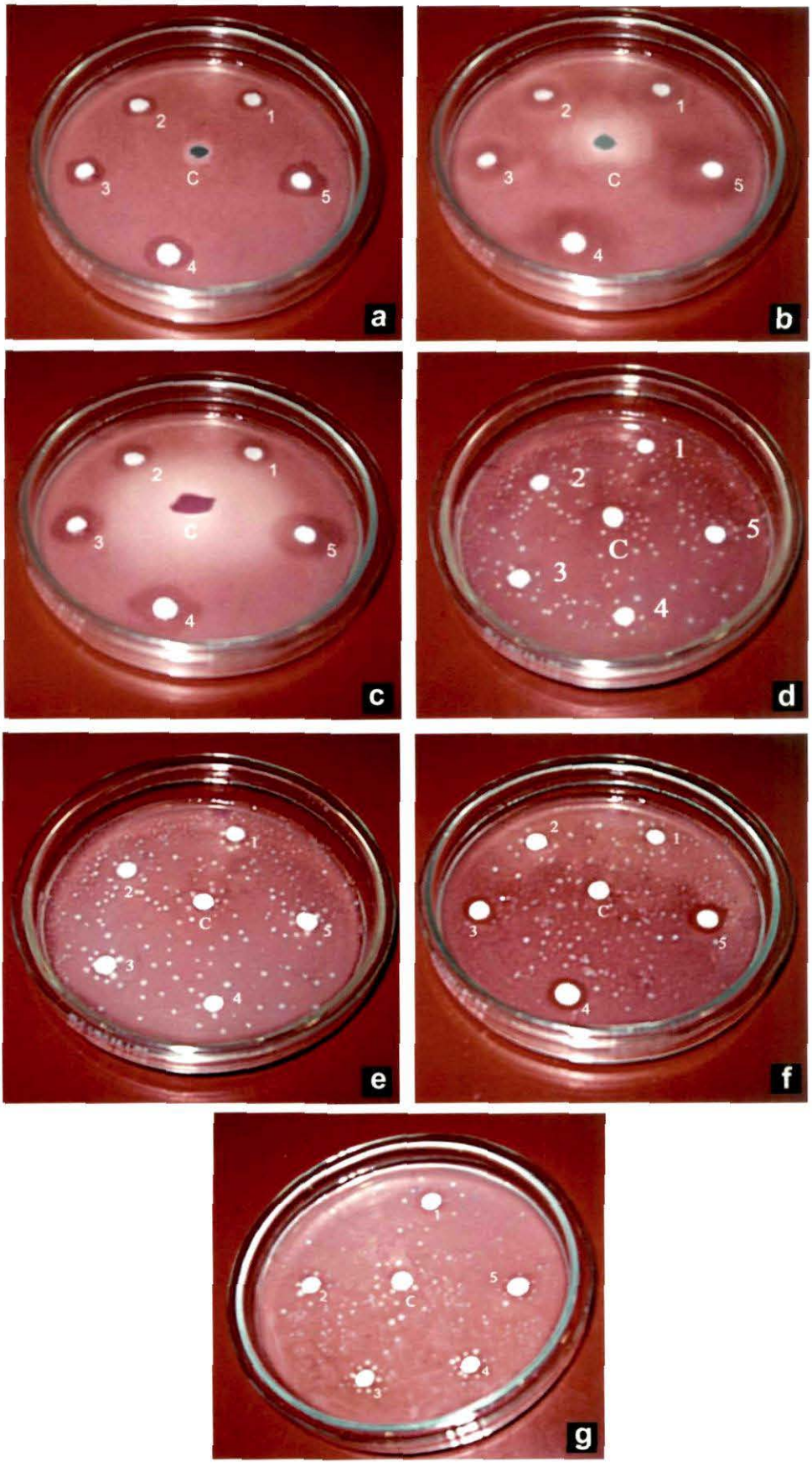


Plate 10: Disc diffusion test for anti microbial activity of compound Cerin, at five different concentrations* against (a) *Colletotrichum gloeosporioides* (b) *Curvularia eragrostidis* (c) *Fusarium equiseti* (d) *Ralstonia solanacearum* (e) *Pseudomonas syringae* (f) *Erwinia carotovora*. (g) *Xanthomonas* sp.

* 1=100ppm, 2=200 ppm, 3=300 ppm, 4=400 ppm, 5=500ppm, c=DMSO control.

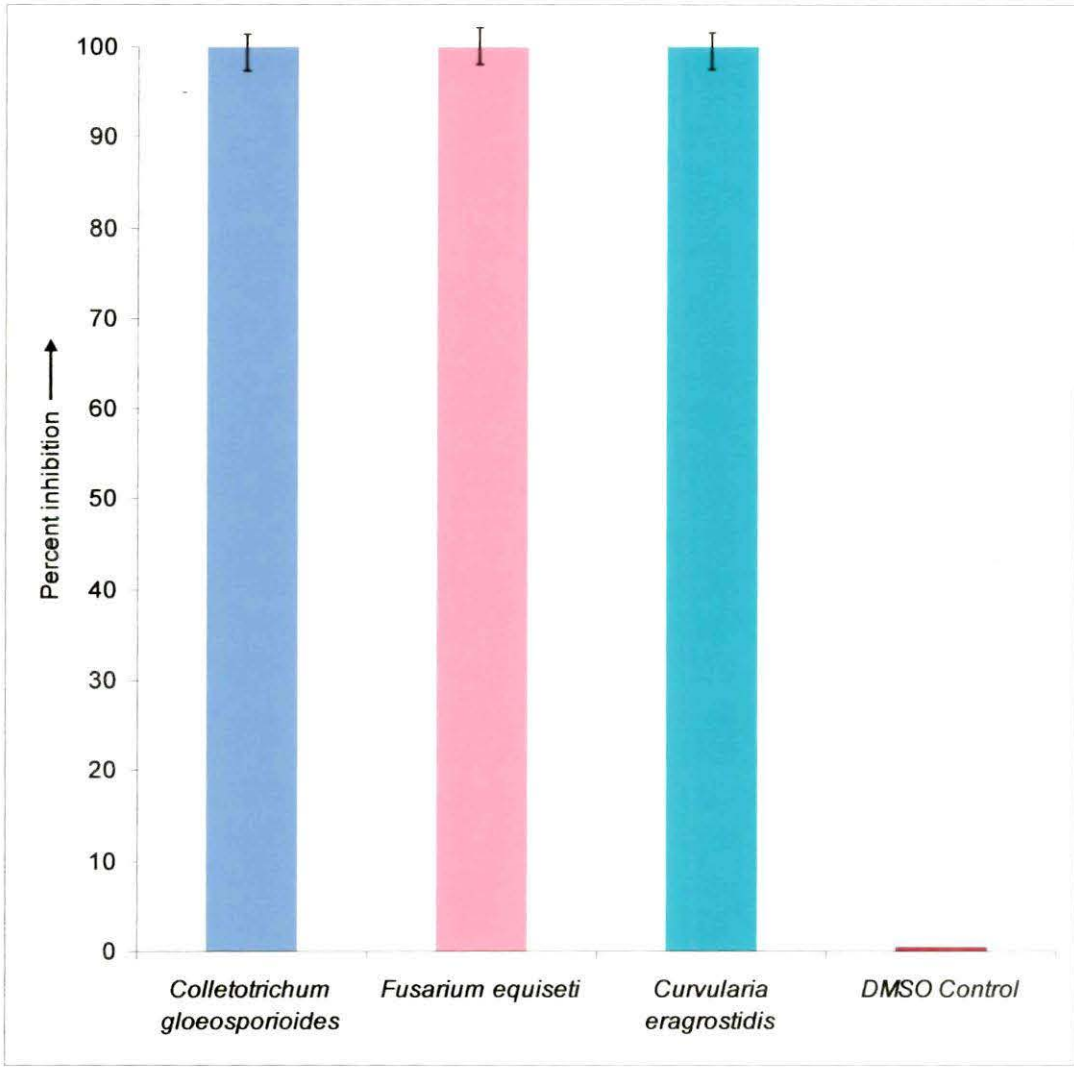


Fig. 37: Percent inhibition of spore germination at 300 ppm concentration of Cerin.

The second compound 'friedelin' was also isolated from cork of *Quarcus suber,* to control some fungal and bacterial pathogens of plants. Five different concentrations of friedelin were used for spore germination bioassay against the three different fungal plant pathogens (*Colletotrichum gloeosporioides, Fusarium equiseti* and *Curvularia eragrostidis*). From the results presented in table 4.4 it was evident that friedelin was also highly antifungal and no spore germination was found to occur even at the lowest concentration (100ppm) tested.

Table 4.4: Percent inhibition of spore germination of *Colletotrichum gloeosporioides, Fusarium equiseti* and *Curvularia eragrostidis* by Friedelin (when control raised to 100).

Fungal organism	Concentrations of compound (ppm)	Range of germ tube length (micrometer)	percent germination	Percent Inhibition*
Colletotrichum gloeosporioides	100	-	-	100
	200	-	-	100
	300	-	-	100
	400	-	-	100
	500	-	-	100
Fusarium equiseti	100	-	-	100
	200	-	-	100
	300	-	-	100
	400	-	-	100
	500	-	-	100
Curvularia eragrostidis	100	-	-	100
	200	-	-	100
	300	-	-	100
	400	-	-	100
	500	-	-	100

*mean of three replications

From the results presented in the table 4.5 & plate 11, it was found that the antifungal activity of friedelin was very high. In disc diffusion tests all the five concentrations of friedelin could effectively control the growth of the fungi as evidenced by larger antifungal zones.

Table 4.5: Antifungal activity of Friedelin

Fungal organism	Concentrations of compounds(ppm)	Diameter of inhibition zone(cm)*
Colletotrichum gloeosporioides	100	0.9
	200	0.9
	300	1.0
	400	1.3
	500	1.4
Fusarium equiseti	100	1.5
	200	1.7
	300	1.8
	400	2.0
	500	2.1
Curvularia eragrostidis	100	1.3
	200	1.5
	300	1.6
	400	1.8
	500	2.0

*mean of three replications; - indicates no inhibition zone formed

Like cerin, friedelin also showed poor antibacterial activity as evidenced from the results presented in table 4.6 and plate-11. In case of *R. solanacearum* and *E. carotovora* two concentrations (400 and 500ppm) of Friedelin showed antibacterial activity. In case of *Xanthomonas sp.* and *P. syringae* bacterial growth was inhibited significantly at concentrations of 300ppm and above.

Table 4.6: Antibacterial activity of Friedelin

Bacterial organism	Concentrations of compound (ppm)	Diameter of inhibition zone(cm)*
Ralstonia solanacearum	Control	-
	100	0.4
	200	0.4
	300	0.4
	400	1.2
	500	1.5
Xanthomonas sp	control	-
	100	0.4
	200	0.4
	300	1.2
	400	1.5
	500	2.0
Pseudomonas syringae	Control	-
	100	0.4
	200	0.4
	300	1.3
	400	1.8
	500	2.0
Erwinia carotovora	Control	-
	100	0.4
	200	0.4
	300	0.4
	400	1.2
	500	1.9

*mean of three replications ; - indicates no inhibition zone formed

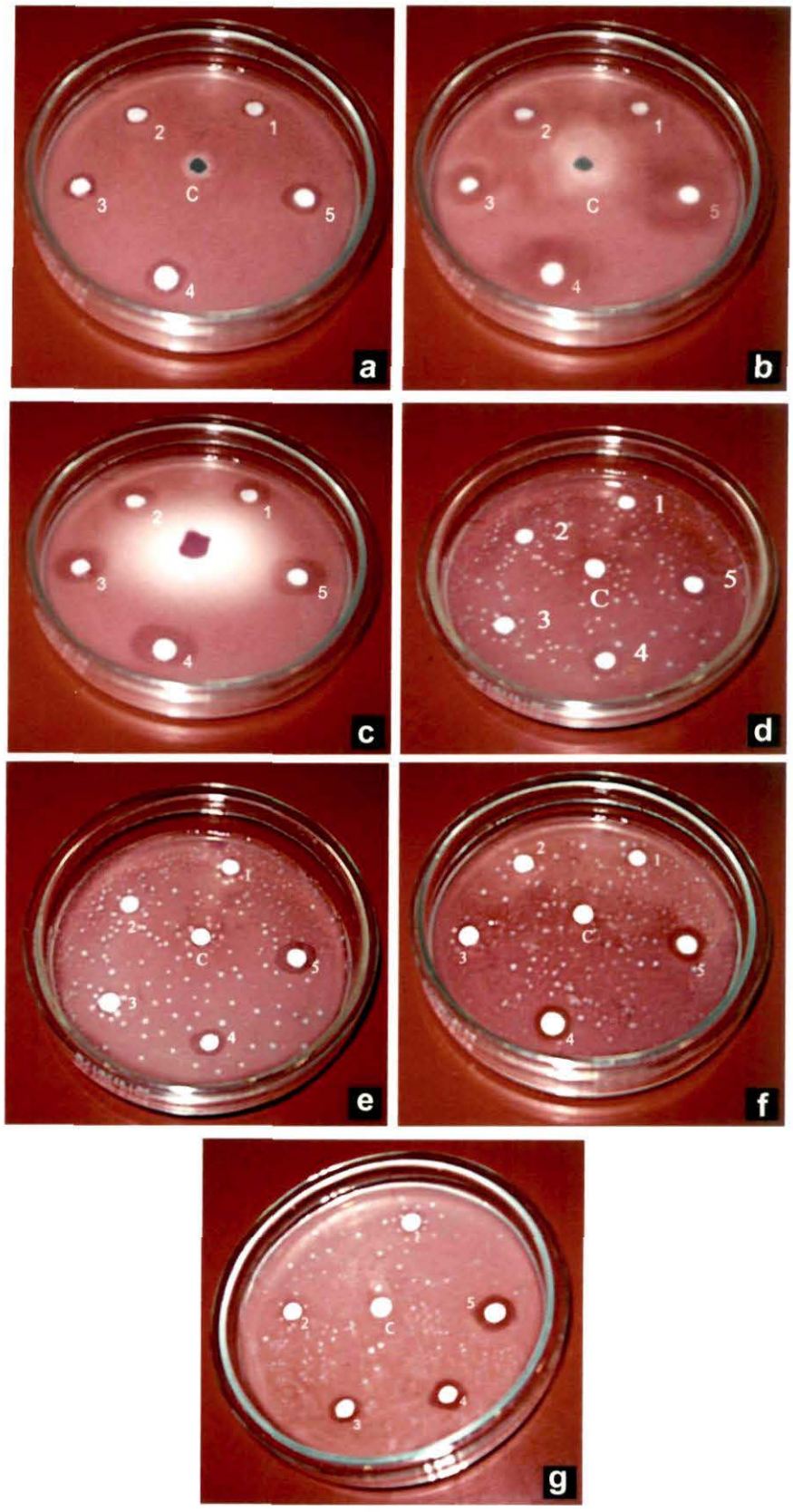


Plate 11: Disc diffusion test for anti microbial activity of compound Friedelin at five different concentrations* against (a) *Colletotrichum gloeosporioides* (b) *Curvularia eragrostidis* (c) *Fusarium equiseti* (d) *Ralstonia solanacearum* (e) *Pseudomonas syringae* (f) *Erwinia carotovora* (g) *Xanthomonas* sp.

* 1=100ppm, 2=200 ppm, 3=300 ppm, 4=400 ppm, 5=500ppm, c=DMSO control.

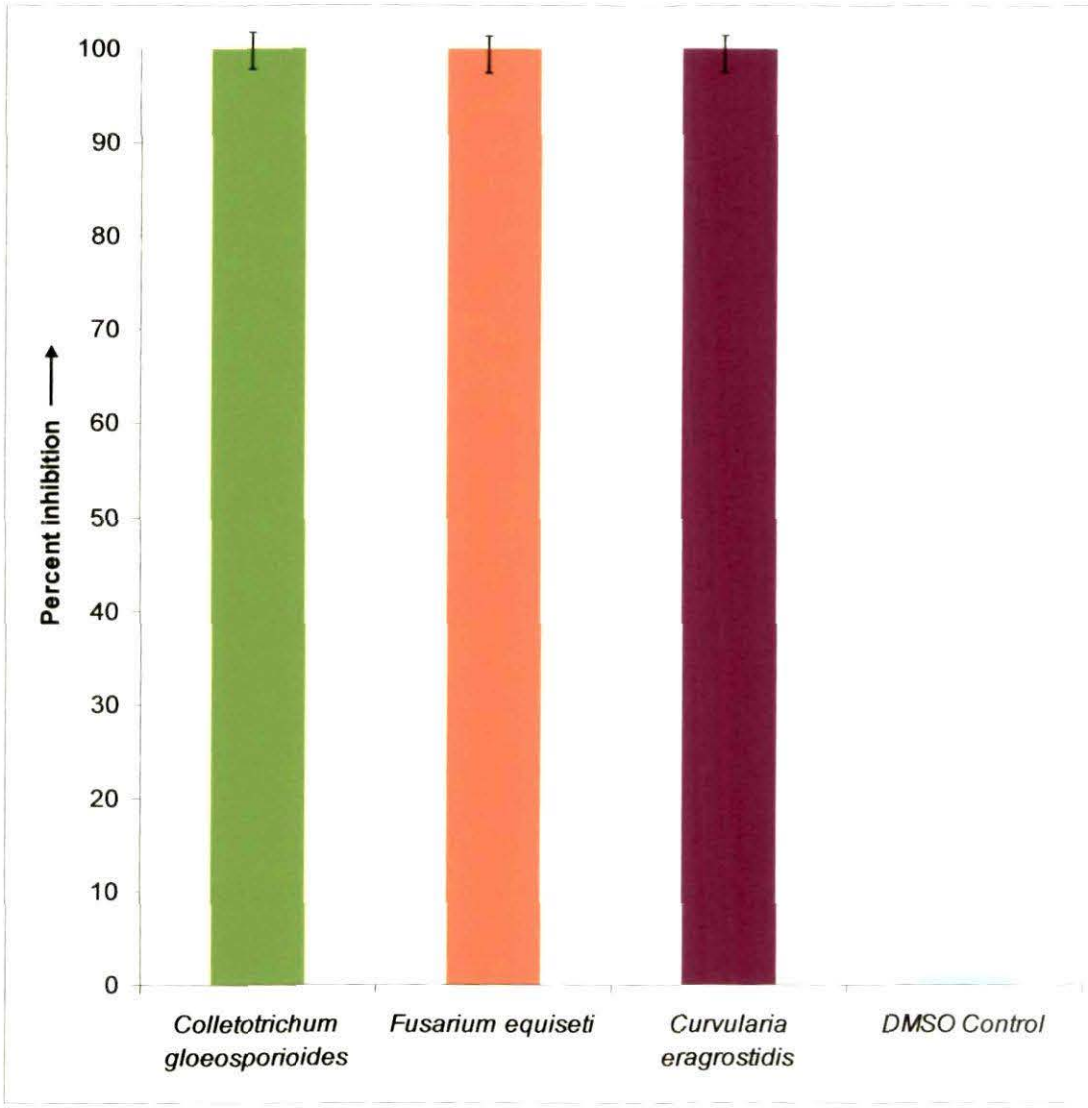


Fig. 38: Percent inhibition of spore germination at 300 ppm concentration of Friedelin.

Phytotoxicity of Cerin and Friedelin:

Phytotoxicity of the two compounds was tested in tomato plants (of Priya variety popularly cultivated in the present study area). To test phytotoxicity, tomato plants were grown in earthen pots [size 15cm (diameter) and 15 cm (height)]. The pots were maintained in the experimental garden of the Department of Botany with normal watering. Thirty such pots containing plants (each 10 cm in height) were kept in a net house. The plants were grouped into three sets and each set contained 10 plants each. Two compounds (at 100 ppm concentration) were sprayed in two sets separately and the third set was sprayed with sterile distilled water. The plants were observed up to seven days. No phytotoxicity was observed at 100ppm concentration of the compounds when compared with the control set which was sprayed with the sterile distilled water. (table-4.7)

Table 4.7: Phytotoxicity of cerin and friedelin at 100ppm concentration

Compounds	Concentrations (ppm)	Morphological & Physiological abnormalities abnormalities.			
		1day	3day	5day	7day
cerin	100	Plants alive, no significant changes Root-germinated Shoot-germinated	Plants alive, no significant changes Root-germinated Shoot-germinated	Plants alive, no significant changes Root-germinated Shoot-germinated	Plants alive, no significant changes Root-germinated Shoot-germinated
friedelin	100	Plants alive, no significant changes Root-germinated Shoot-germinated	Plants alive, no significant changes Root-germinated Shoot-germinated	Plants alive, no significant changes Root-germinated Shoot-germinated	Plants alive, no significant changes Root-germinated Shoot-germinated
Control	Sterile distilled water	Plants alive, no significant changes Root-germinated Shoot-germinated	Plants alive, no significant changes Root-germinated Shoot-germinated	Plants alive, no significant changes Root-germinated Shoot-germinated	Plants alive, no significant changes Root-germinated Shoot-germinated

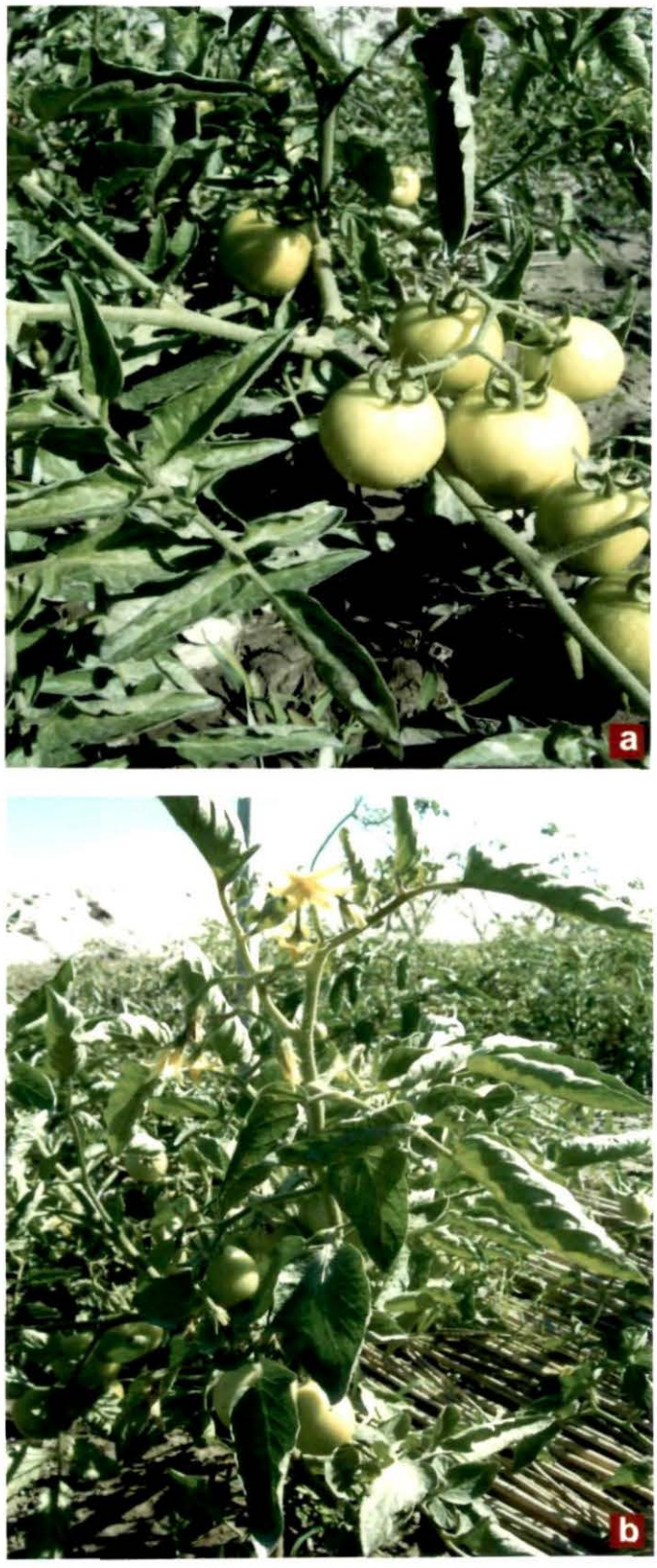


Plate 12: Tomato plants used in different *In vivo* studies (Priya variety)

2.3: Discussion

In Chapter IV isolation, characterization and preparation of two antimicrobial derivatives from the plant *Quarcus suber* have been described. It is a plant commonly called Cork oak belongs to the family Fagaceae [1]. Through phytochemical extraction of *Quarcus suber*, two derivatives Cerin and Friedelin have been prepared from dried and finely powdered cork, extracted with petroleum ether in a Soxhlet apparatus for 72 hours and was characterized. Cerin and Friedelin, both the derivatives were very significant to inhibit the spore germination of fungi (*Colletotrichum gloeosporioides, Fusarium equiseti and Curvularia eragrostidis).*

From the results of 'spore germination bioassay' cerin showed its highly antifungal activity. No spore germination was observed at any of the five concentrations (100, 200, 300, 400 & 500ppm) tested. Lowest concentration (100ppm) of the compound showed cent percent inhibition of spore germination. In disc diffusion method mycelial growth of the three fungi (*Colletotrichum gloeosporioides, Fusarium equiseti and Curvularia eragrostidis*) was also controlled by Cerin significantly even at 100ppm concentration. In contrast to the antifungal activity, Cerin could not show its efficacy towards the three bacteria (*R. solanacearum, Xanthomonas sp.* and *P. syringae*). Growth of *E. carotovora* was controlled by Cerin only at higher concentrations (300,400,500ppm) as evident from disc diffusion test (table-4.3). Friedelin, like the cerin did not allow the spores of the three fungi (*Colletotrichum gloeosporioides, Fusarium equiseti* and *Curvularia eragrostidis*) to germinate at lowest concentration tested. Results of the disc diffusion test also showed significant antifungal activity of the compound. Friedelin, in comparison to Cerin, showed smaller diameter of inhibition zones in disc diffusion method. Friedelin also showed insignificant antibacterial activity at lower concentrations (100 & 200ppm) but all the four bacteria (*R. solanacearum, E. carotovora Xanthomonas* sp. and *P. syringae*) tested was controlled by Friedelin at higher concentrations (400 & 500ppm). Thus friedelin was more antifungal than antibacterial.

In several respects our results were supported by different workers. Ramesh *et al.* (2008) isolated Friedelin, epi-Friedelin, n-Octacosanol, α-Amyrin, Sitosterol, Sitosterol-3-α-D-glucopyranoside and luteoforol from *Bridelia crenulaa* Roxb. The aqueous and

methanolic extracts and their fractions were tested against ten human pathogenic bacteria and four fungal strains. They observed that inhibitory activities were maximum in the chloroform-methanol (1:1) fraction of the methanolic extract against *E.coli, K.pneumoniae* and *P.aeruginasa*, which were responsible for the pathogenesis of urinary tract infection. Their study provided scientific evidences for the efficacy of the use of triterpedoids. Khan *et al.* (2008) extracted crude from the leaves, stem bark, stem heart wood, root bark and root heart wood of *Euroschinus papuanus* and isolated fractions on partitioning with petrol, dichloromethane, ethyl acetate and butanol and studied antibacterial and antifungal activity. They observed that ethylacetate fractions of the stem heart wood, dichloromethane fraction of root bark and butanol fraction of root heart wood exhibited excellent antibacterial activity and butanol fraction of leaves, stem heartwood and root bark exhibited antifungal activity. Prusky *et al*, (1982) and Rahmani *et al* (2006) reported that leaf extract of *Datura metel, X. strumarium* exhibited 100% of inhibition of spore germination of *A.alata*. Spore germination of *P.theae* was completely inhibited by leaf extract of *X.strumarium* and *D.stamoniun*. The antifungal potentiality of several plant extracts through spore germination bioassay were reported by the authors. Ramesh *et al* (2002) performed a phytochemical investigation of the various extracts of the leaves of *Begonia malabarica* L. (Begoniaceae) resulted in the isolation and identification of six known compounds, viz. friedelin, epi-friedelinol, beta-sitosterol, luteolin, quercetin and beta-sitosterol-3-beta-D-glucopyranoside. The aqueous and organic solvent extracts were also tested against ten human pathogenic bacteria and four fungal strains by the agar-well diffusion method. All the extracts were devoid of antifungal activity against the tested fungi. The hexane extract did not show any activity. The aqueous extracts showed activity against the Gram-negative bacteria except *Vibrio parahaemolyticus*. The chloroform and methanol extracts showed activity against all the tested bacteria.

Duraipandiyan *et al* (2006) designed an experiment to evaluate the antifungal activity of *Azima tetracantha* extracts and isolated compound friedelin and used against fungi. Antifungal activity was carried out using broth micro dilution method and fractions were collected using (silica gel) column chromatography. The antifungal activity of *Azima tetracantha* crude extracts and isolated compound (friedelin) were evaluated using

the micro dilution method. Hexane extract showed some antifungal activity. The compound also exhibited antifungal activity against same fungi. They showed lowest MIC against *Trichophyton rubrum* and *Curvularia lunata.* In both the cases it was 62.5 μg/ml. They suggested that Friedelin was a promising antifungal agent. Shing *et al.* (2001) reported that the hexane extract of *Heliotropium marifolium* yielded a mixture of triterpenoids: β-sitosterol, stigmasterol, β-amyrin, friedelan-3β-ol (epifriedelenol), cycloartenone, β-amyrin acetate, friedelin and epifriedenyl acetate. Isolated triterpenoid and reference antibiotics (gentamycin/mycostatin) were tested against selected pathogenic bacteria and fungi, e.g. *Escherichia coli, Staphylococcus aureus, Aspergillus niger* and *Penicillium chrysogenum.* Joseph *et al* (2010) reported that the 50% methylene chloride in hexane fresh column fraction of the extract of leaves of *Ficcus racemosa* was found to be antifungal. The extract inhibited the growth of several plant pathogens (*Curvularia sp, Colletotrichum gloeosporioides, Alternaria sp, Fusarium sp*).

Phytotoxicity test of cerin and friedelin at 100ppm concentration showed no toxicity on the plants tested up to seven days. Hence the compounds which were effective in controlling pathogens at 100ppm concentration may be recommended for controlling the pathogens *in vivo* or in field conditions.

SECTION 3: References

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5. Khan M.R.; Omoloso A.D. and Kihara M. (2004). Antibacterial and antifungal activities of *Euroschinus papuanus. Fitoterapia.* **75:** 412-416.
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8. Singh B. and Dubey M.M. (2001). Estimation of triterpenoids from *Heliotropium marifolium in vivo* and *in vitro* antimicrobial screening. *Phytotheraphy Research.* **15 :** 231-234.
9. Joseph B. and Raj S.J. (2010). Phytophermacological properties of *Ficus racemosa* Linn. –an-overview. *International Journal of Pharmaceutical Sciences.* **3:**134-138.

Conclusion

From the results of the present study and the works done by several workers, as stated above it may be concluded that some natural triterpenoids and their derivatives may be used to check specific plant pathogens after phytotoxicity test *in vivo*. Therefore, the outcome of the investigation not only would enrich the understanding of structure and their biological activities among the four types of natural triterpenoid groups (Lupeol, betulinic acid, cerin and friedelin) and some of their derivatives, but at the same time would provide a scientific base to control plant pathogens. Implication of the results was discussed in the respective discussion portions of section-2 of the chapters II, III and IV. The results were encouraging since natural triterpenoid compounds and their derivatives were potential to control fungal and bacterial pathogens. On the basis of phytotoxicity tests, it was found that 100ppm concentration of all the 11 compounds were not phytotoxic(result showen).At higher concentrations(200ppm-500ppm) the compounds were phytotoxic. From the present study, it was observed that several plant pathogens may be controlled by the compounds tested. But best antimicrobial activity differed for different pathogens. Considering all the evidences of experimental tests it was found that best control may be achieved by compound-F and Cerin in case of *Colletotrichum gloeosporioides*. Similarly, *Fusarium equiseti* may be best controlled by compound-G and Cerin. The third fungi, *Curvularia eragrostidis* and *two bacteria* (*Ralstonia solanacearum* and *Erwinia carotovora*) may be best controlled by compound-B. In case of controlling *Xanthomonas sp* compound-B and –F were proved to be best. The seventh pathogen, *Pseudomonas syringae* was significantly controlled by compound-H.

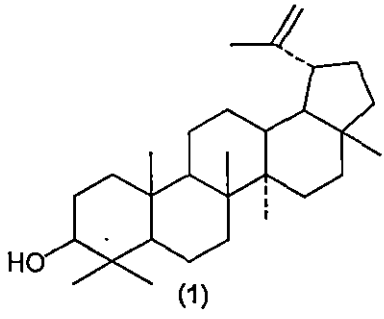
SUMMARY

The investigation embodied in the present thesis entitled "Studies on the antimicrobial activity of some available triterpenoids and its derivatives from some medicinal plants" was initiated under the supervision of Dr. A. Saha, Department of Botany and Dr. P.Ghosh, Department of Chemistry, University of North Bengal, Siliguri,India. The study consisted of: (i) Isolation and characterization of natural compounds. (ii) Preparation of derivatives from natural compounds, their characterization and screening of their antimicrobial activity against some well known fungal and bacterial pathogens of economically important plants. In the present work three medicinal plants (*Xanthoxylum budrunga, Bischofia javanica* and *Quarcus suber*) were selected for extraction of triterpenoids.

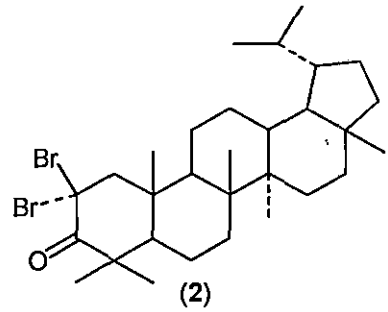
Following a short Introduction, the whole work was divided into four chapters. **Chapter-I** dealt with a comprehensive review of literature in concord with the present line of investigations. **Chapter-II** dealt with *Xanthoxylum budrunga* and contained two sections. **Section-1** described details of the isolation and characterization techniques of the compounds (including derivatives) with required experimental data, their analyses and discussions. **Section-2** dealt with antimicrobial efficacy of the compounds. **Section-2** included materials and methods, results and discussion in details. **Chapter-III** dealt with the plant *Bischofia javanica* and **Chapter-IV** dealt with the plant *Quarcus suber.* Like **Chapter-II the Chapter-III** and the **Chapter-IV** each was consisted of two sections.

Chapter-II (section-1): *Xanthoxylum budrunga* was selected to isolate lupeol. Triterpenoid, Lupeol was isolated and characterized. Antimicrobial assay of the compound was performed against some fungal and bacterial plant pathogens. Lupeol, the mother compound (triterpenoid) was coded as compound-A and was isolated with chloroform in soxhlet apparatus. several fractions were obtained from the soxhlet. From those fractions, some derivatives were prepared by the processes like Jone's oxidation method, treatment with N-bromosuccinimide, dehydrobromination of 2α-bromolupanone with lithium bromide-N, N-dimethylformamide, treatment of lithium-ethylenediamine and microwave assisted cyclisation of dioxime. The derivatives were commonly called as 2,2-dibromolupanone (coded as compound-B), 2α-bromolupanone (coded as compound-

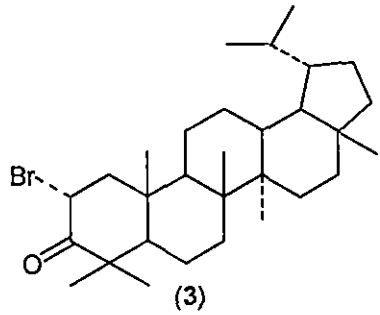
C), lup-1(2)-en-3-one (coded as compound-D), and Lupan[2,3-C]-1′,2′,5′-oxadiazole (coded as compound-E). The structure of these compounds was determined by chemical and spectral data in comparison with that of spectral data of already reported compounds. The structures of the compounds are as follows:



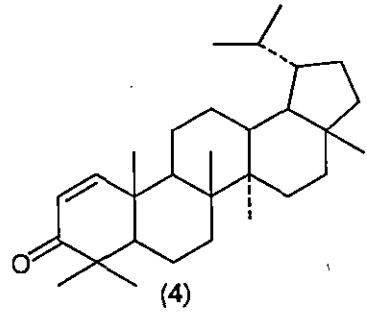
(1)
Lupeol



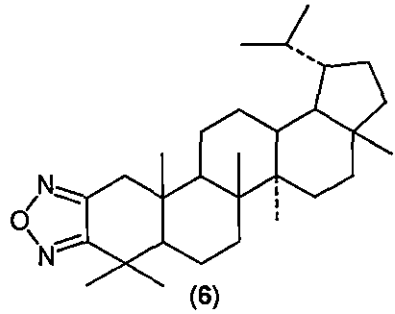
(2)
2,2-dibromr lupanonel



(3)
2α-bromr lupanonel



(4)
Lup-1(2)-en-3-one

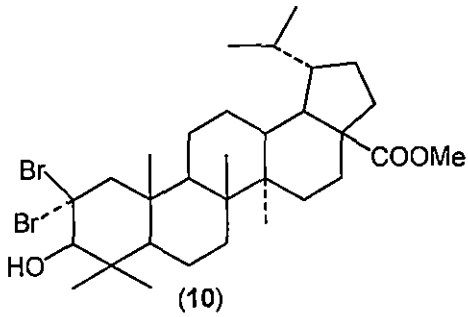


(6)
Lupane-[2,3-C]-1′,2′,5′-oxadiazole

Chapter-II (Section-2): Finally, the mother compound lupeol and its five derivatives (in five different concentrations viz.100, 200, 300, 400 & 500ppm) were subjected to

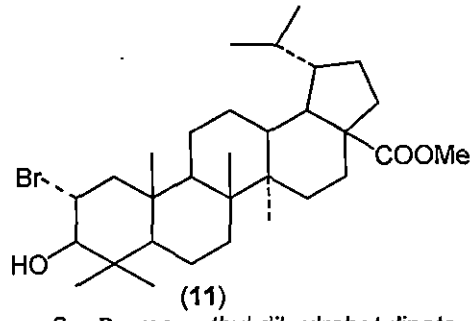
bioassays against three fungal and four bacterial plant pathogens. Materials and methods, Results and discussions related to the antimicrobial efficacy of the compounds were also described in details in this section.

Chapter III (section-1) dealt with the plant *Bischofia javanica* which belongs to the family Euphorbiaceae. Betulinic acid, the natural triterpenoid was isolated from the trunk bark of the plant and was characterized. Like chapter-II some derivatives of betulinic acid were also prepared following procedures like estarification of betulinic acid, Hydrogenation of methyl betulinate, Jone's oxidation of lupanol, and treatment of methyldihydrobetulonate with N-bromosuccinimide, dehydrobromination of 2α-bromodihydromethylbetulonate, and cyclisation of dioxime. The obtained derivatives were 2,2 dibromomethyldihydrobetulonate (coded as compound-F), 2α-bromomethyl-dihydrobetulonate (coded as compound-G), 28 carbomethoxylup-1(2)-en-3-one(coded as compound-H) and 28 carbomethoxylupan-[2,3-C]-1′,2′,5′-oxadiozole (coded as compound-I). Structures of the compounds are as follows:



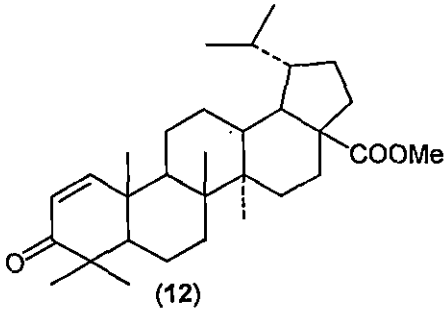
(10)

2,2-Dibromomethyl dihydrobetulinate



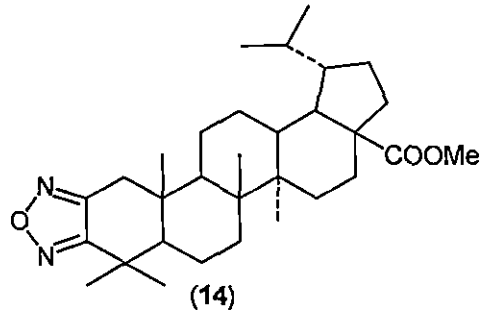
(11)

2α–Bromomethyl dihydrobetulinate



(12)

28-Carbomethoxy lup-1(2)-3-one

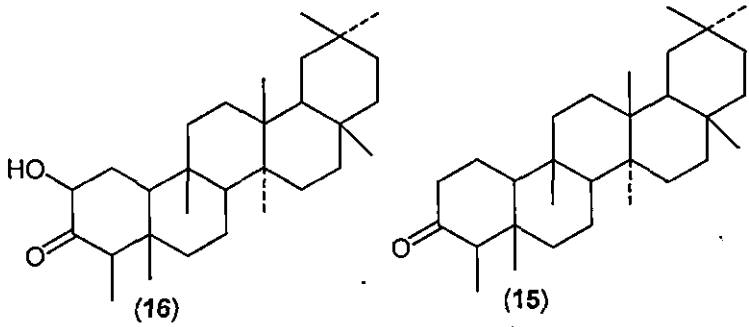


(14)

28-Carbomethoxy lupan [2,3-C]-1′,2′,5′-oxadiazole

Chapter III (section-2): Here, the mother compound betulinic acid and its three derivatives (in five different concentrations viz.100, 200, 300, 400 & 500ppm) were subjected to bioassays against the same three fungal and four bacterial plant pathogens. *Materials and methods, Results and discussions related to the antimicrobial efficacy of* the compounds were also described in details in section-2 of chapter II.

Chapter IV (section-1) dealt with the plant *Quarcus suber* which belongs to the family Fagaceae. Two derivatives Cerin and Friedelin were prepared from the cork of the plant. Detailed extraction process was given in this section of the chapter IV. Structures of the compound are as follows;



(16) Cerin **(15)** Fridelin

Chapter IV (section-2): In this section antifungal and antibacterial activity were performed by the two compounds (Cerin and Friedelin) in five different concentrations (100,200,300,400,500ppm) as in the other cases done. The same three fungal pathogens and four bacterial pathogens were used to test the antifungal efficacy of the two compounds. Details of the materials and methods, results and discussion related to the antimicrobial efficacy of the compounds were also included in this section. On the basis of the experimental evidences, Friedelin proved to be more effective than cerin.

Implication of the results was discussed in the respective discussion portions of section-2 of the chapters II, III and IV. The result was encouraging since natural triterpenoid compounds and their derivatives were potential to control fungal and bacterial pathogens. On the basis of phytotoxicity tests, it was found that 100ppm concentration of all the 11 compounds were not phytotoxic. From the present study, it has been observed that several plant pathogens can be control by the compounds tested which

are effective in controlling pathogens at 100 ppm concentration and are also phytotoxic may be recommended for controlling the plant pathogens *in vivo* or in field condition. Considering all the evidences of experimental data, it can be concluded that *Colletotrichum gloeosporioides*-may be controlled by compound F and Cerin , *Fusarium equiseti*— may be controlled by compound G and Cerin, *Curvularia eragrostidis*- may be controlled by compound B, *Ralstonia solanacearum*- may be controlled by compound B, *Xanthomonas sp*- may be controlled by compound B and F,*Pseudomonas syringae*- may be controlled by compound H, *Erwinia carotovora*-- may be controlled by compound B.