1996

Studies on the Effect of Extractions of Different Parts of Amoora Sp. on the red Flour Beetle, Tribolium Castaneum (HBST.)"

Islam, Md. Nurul

STUDIES ON THE EFFECT OF EXTRACTIONS OF DIFFERENT PARTS OF *AMOORA* SP. ON THE RED FLOUR BEETLE, *TRIBOLIUM CASTANEUM* (HBST.)

A THESIS
SUBMITTED TO THE UNIVERSITY OF RAJSHAHI
IN FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF

DOCTOR OF PHILOSOPHY

BY

Md. Nurul Islam

INSTITUTE OF BIOLOGICAL SCIENCES
RAJSHAHI UNIVERSITY
BANGLADESH

SEPTEMBER, 1996

Prof. M. Khalequzzaman
B.Sc. (Hons), M.Sc., Ph.D., FRES (London)
Chairman

Department of Zoology
University of Rajshahi
Rajshahi 6205
Bangladesh

Certificate

It is my pleasure to certify that the thesis entitled, "STUDIES ON THE EFFECT OF EXTRACTIONS OF DIFFERENT PARTS OF *AMOORA* SP. ON THE RED FLOUR BEETLE, *TRIBOLIUM CASTANEUM* (HBST.)" submitted by Mr. Md. Nurul Islam in fulfilment of the requirements for the degree of Doctor of Philosophy in Crop Protection and Toxicology in the Institute of Biological Sciences, Rajshahi University, is a dissertation of the perfect study which he carried out partly in Bangladesh and partly in Switzerland with much success. It contains no materials previously published or written by any other person except, wherever, due references are made in the text of the thesis.

I hereby clarify that the author completed his work mostly under my direct supervision and partly under Prof. Kurt Hostettmann, Chef de l'Institut de Pharmacognosie et Phytochimie, Ecole de Pharmacie, Université de Lausanne, CH-1015 Lausanne-Dorigny, Suisse/Switzerland and contributed some new ideas and openings in our research field by adding modern technologies of the contemporary world.

Dated : September, 1996
Rajshahi

(M. Khalequzzaman)

Telephone (880) 721-3041-9 Ext.-423 (Office)
(880) 721-4004 (Residence)
Fax. (880) 721-2064

UNIVERSITE
DE
LAUSANNE

INSTITUT DE PHARMACOGNOSIE
ET PHYTOCHIMIE

dir.: Prof. K. Hostettmann

ATTESTATION

This is to certify that ***Md. Nurul Islam*** *date of birth 02.11.1966 of the Institute of Biological Sciences, Rajshahi University, Bangladesh, was admitted in my institute as a Post-graduate student to complete a part of the substantial work for his Ph.D. under my supervision. During his stay in my laboratories he carried out bioassays on Cladosporium cucumerinum, Aedes aegypti, Candida albicans, Bacillus subtilis, and Biomphalaria glabrata with the leaf, seed, fruit-pericarp, bark and root extracts of Amoora rohituka W. & A. extracted in petroleum ether, ethyl acetate, acetone and methanol. He isolated two compounds from the leaf extract successfully.*

It is permitted to add this part of his work in his dissertation for Ph.D.

K. Hostettmann

Professor Kurt HOSTETTMANN
Director
Institute of Pharmacognosy and Phytochemistry
University of Lausanne
SWITZERLAND

Dated: 15.12.95
Lausanne-Dorigny

Ecole de Pharmacie, BEP, CH-1015 Lausanne-Dorigny (Suisse)
Téléphone (021) 692 28 61, Fax (021) 692 28 80

Dedicated
to my parents

Acknowledgment

I wish to express my indebtedness with heartiest gratitude to my honorable teacher and supervisor Dr. M. Khalequzzaman, Professor and Chairman, Department of Zoology, Rajshahi University, Bangladesh; for his continued interest, stimulating discussion and valuable suggestions during the supervision of this work. I am especially grateful to him for being introduced with the techniques of phytochemical extraction that he studied during his Post Doctoral Research at the University of Newcastle upon Tyne, England.

My deepest appreciation is extended to my supervisor in abroad Professor Kurt Hostettmann, (Institut de Pharmacognosie et Phytochimie, Ecole de Pharmacie, Université de Lausanne, Suisse;) who opened door for my research abroad to be well acquainted with modern techniques of isolation, purification and characterization of naturally occurring secondary metabolites used in the disease and pest control strategies. I would also like to pay best regards to my technical advisor Dr. Olivier Potterat, (Assistant Professor, Institut de Pharmacognosie et Phytochimie, Ecole de Pharmacie, Université de Lausanne, Suisse,) who actually guided me for isolation procedures in practice.

I wish to express my deepest sense of gratitude to the Director of the Institute of Biological Sciences, Rajshahi University, for offering Fellowships and for providing laboratory facilities during the course of this work.

It is not a courtesy rather an obligation to acknowledge that the whole work was supported financially partly by the Institute of Biological Sciences, Rajshahi University, Bangladesh (provided a three year Fellowship); and partly by The Swiss Federal Commission for Scholarships for the Foreign Students (provided a Fellowship for one year and six months) under assistance by The SDC (The Swiss Development Cooperation) in Bangladesh under care of the Embassy of Switzerland in Dhaka. The Ministry of Finance of the Peoples Republic of Bangladesh is also acknowledged for the grant from the Bangladesh-Swedish Trust Fund.

Heartiest remembrance and sincere thanks to all my teachers of the Department of Zoology, Rajshahi University, together with Madam M. Hostettmann, Dr. Andrew Marston, Dr. Marc Maillard, Dr. Jean-Luc Wolfender of the Institute of Pharmacognosy and Phytochemistry, University of Lausanne, Madam Dubois, Madam Islar and all of the UNIL office staff, Lausanne-Dorigny, Switzerland, Monsieur Guy Dudan of Ciba Geigy ltd., Prof. Benny Tan of the Department of Pharmacology, National University of Singapore, Singapore, Dr. Anwarul Islam and Prof. Muhd. Ekramul Haque of the Department of Pharmacy, Prof. Azizul Islam of the Department of Chemistry, Dr. M. Monzur Hossain and Mr. M.I. Waheed of Department of Botany, Mrs. U.M. Rahela Khatun and all of the I.Bio.Sc. office staff and Mrs. A. J. Ismat Ara Chowdhury of the Department of Zoology, Rajshahi University, Bangladesh, for their continuous help, support and encouragement.

I would like to remember all my fellow workers, especially friends (in home-) Rhita, Era, Nuruzzaman, Aravinda Mondal, Provash Biswas, Shewli, Shahiquzzaman, Parvez Hassan, Shahinul Islam, Asif Hasan, [*too many!*] and (in abroad-) Chantal Bergeron, Stefan Gafner, Guido Rath, Muriel Cuendet, Fabrizio Componovo, Olga Maria Duarte Silva, Cecilia Cechhini, Bertrand Ducrey, Cremin Peadar, Nicole Hytte, Dãsa Zagreb, Caroline Keller, Nicola Fuzzati, Ma Wei-Guang, Ren-Xiyang Tan, Pilar, Vien, Cécé, Eliane Garo, François Miserez, Jean-Robert, Raja, Leandro, Philippe, Alexandre, Sylvain Rodriguiez and Christian Terreaux, without whom this work could not have been carried out, or without whom it might have been done sooner.

I am thankful to my family for their sacrifice with almost five years of patience leaving me potentially detached to have enough concentration in carrying out this work successfully.

Mr. Nitai Kumar Mondal, Mr. Monzur Ahmed and Mr. Asaduzzaman are thanked for helping me in typing and making the dissertation complete with sincerity and devotion.

The Author

Abbreviation of the special words used in the text

1,2	:	Symbols used to mention compounds
A, B, A_1, B_1	:	Symbols used to mention isolated batches in fractionation
a,b,c	:	Symbols used to mention compounds reported by previous workers
BA	:	Stem-bark extract in acetone
BE	:	Stem-bark extract in ethyl acetate
BM	:	Stem-bark extract in methanol
BP	:	Stem-bark extract in petroleum spirit
b.p.	:	Boiling point
COSY	:	NMR spectrum of homonuclear correlations ^1H-^1H
dec.	:	Decomposition
DEPT	:	Distorsionless Enhancement by Polarization Transfer
d.i.	:	Internal diameter
DMSO	:	Deuteromethyl sulphoxide
ED_{50}	:	Effective Dose 50
EI	:	Electronic impact
EtOAc	:	Ethyl acetate
Fig.	:	Figure
Fr.	:	Fraction
h	:	Hour/hours
HPLC	:	High performance/High-pressure liquid chromatography
HPTLC	:	High potential thin layer chromatography
in vacuo	:	With vacuum

IR	:	Infrared
LA	:	Leaf extract in acetone
LC_{50}	:	Lethal concentration 50
LD_{50}	:	Lethal dose 50
LE	:	Leaf extract in ethyl acetate
LM	:	Leaf extract in methanol
LP	:	Leaf extract in petroleum spirit
M+	:	Mass
M/z	:	Mass for electronic charge
MS	:	Mass spectrum
MeOH	:	Methanol
mg	:	Milligram
ml	:	Milliliter
m.p.	:	Melting point
nm	:	Nanometre
µg	:	Microgram
µg/µl	:	Microgram per microlitre
µl	:	Microlitre
µm	:	Micrometre
NMR	:	Neuclear Magnetic Resonance of proton ^{1}H and ^{13}C
OD_{600}	:	Optical density at 600 nm
P-388	:	Murine P-388 lymphocytic leukemia cell line
Pet. ether	:	Petroleum ether
Pet. spt.	:	Petroleum spirit

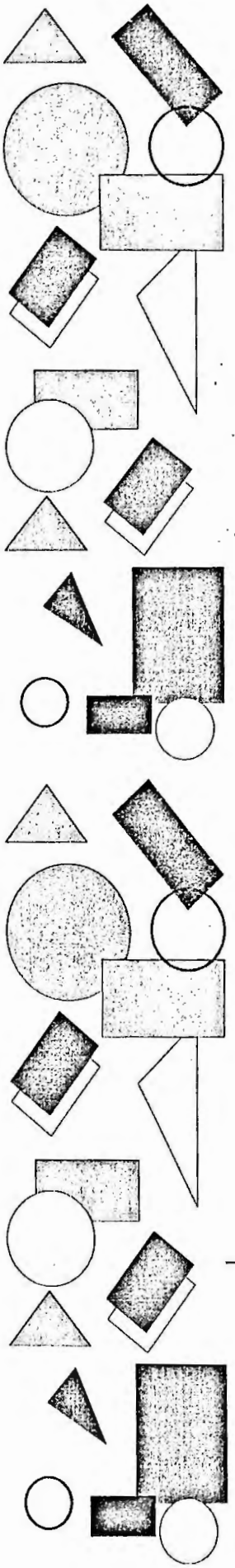
RA	:	Root extract in acetone
RE	:	Root extract in ethyl acetate
Rf.	:	Relative frequency
RM	:	Root extract in methanol
Rotavapor	:	Rotary evaporator
RP	:	Reversed phase
RP	:	Root extract in petroleum spirit
rpm	:	Rotation per minute
SA	:	Seed extract in acetone
ScA	:	Seed-coat (Fruit-Pericarp) extract in acetone
ScE	:	Seed-coat (Fruit-Pericarp) extract in ethyl acetate
ScM	:	Seed-coat (Fruit-Pericarp) extract in methanol
ScP	:	Seed-coat (Fruit-Pericarp) extract in petroleum spirit
SE	:	Seed extract in ethyl acetate
SM	:	Seed extract in methanol
SP	:	Seed extract in petroleum spirit
Tab.	:	Table
TLC	:	Thin layer chromatography
TMS	:	Tetramethylsilane
UV	:	Ultraviolet
Wt.	:	Weight
χ^2	:	Chi squared

A checklist of the figures provided

A checklist of the tables provided

A checklist of the charts provided

CONTENT



Chapter 1

General Introduction

Chapter 1 General Introduction

Plants are considered to be the most potent objects to human beings not only because of their support for food and shelter, but also because they provide almost all the requirements for the survival of the civilization. Within the past few decades the world advanced rapidly with remarkable development in pesticide technology and medicine, but there are still some problems especially in the field of pesticides for undesirable changes in the gene-pool for the presence of some mutagenic agents and also for increasing pesticide resistance in insects. So, a question has arisen for the sustainability and the survivability of the living beings on the planet with non-hazardous environment. Hence a worldwide interest has created in the reevaluation and use of age-old traditional botanical agents (Heyde *et al.*, 1983).

Until now only a small part of the plant kingdom (estimated at 2,50,000-5,00,000 species around the globe) has been investigated phytochemically and the fraction subjected to biological and pharmacological screening is even lower. Since plants may contain hundreds or even thousands of metabolites, there is currently a resurgence of interest in the vegetable kingdom as a possible source of new lead compounds for introduction into therapeutical screening programmes (Hostettmann *et al.*, 1995).

Moreover botanicals, because of their low mammalian toxicity, have received much attention as control agents against stored grain pests. According to Feinstein (1952) over 2000 species of plants representing 170 odd families are said to have some insecticidal values. There are many species of plants of these families in Bangladesh which are used as traditional medicine by the native people from the remotest antiquity.

In the agricultural sector awareness has created for continuous or heavy use of some pesticides which caused serious problems arising from factors, such as, direct toxicity to parasites, predators, pollinators, fish and man (Munakata, 1970; Pimental, 1981), pesticide resistance (Brown, 1968; Georghiou and Taylor, 1977; Schmutterer, 1981; Waiss *et al.*, 1981), susceptibility of crop plants to insect pests (Pimental, 1977), and increased environmental and social costs (Pimental *et al.*, 1980), genetic resistance of pest species, toxic residues, increasing costs of application, environmental pollution, hazards from handling, etc. (Ahmed *et al.*, 1981), cross and multi-resistant strains in many important insect species (Dyte, 1970; Pasalu and Bhatia, 1983; Dyte and Halliday, 1985; Irshad and Gillani, 1990; Zettler and Cuperus 1990; Zettler, 1991). It has, therefore, become necessary to complement our reliance on synthetic pesticides with less hazardous, safe and biodegradable substitutes. Recently, the search for naturally occurring antifeedants against pests of yield crops and storage has been intensified. A number of investigators isolated, identified and screened compounds from parts of many botanical families for insect feeding deterrence and growth inhibition (Jacobson *et al.*, 1975; Bernays and Chapman, 1977; Doskotch *et al.*, 1977; Jacobson, 1977; Shudhakar *et al.*, 1978; Carpentier *et al.*, 1979; Warthen, 1979; Jurd and Manners, 1980; Menn, 1980; Saxena, 1983). Locally available plants and minerals have been widely used in the past to protect stored products against damage by insect infestation (Golob and Webley, 1980). The main advantage of botanicals is that they are easily produced by farmers in small-scale industries and are potentially less expensive. Moreover, botanical insecticides are

broad-spectrum in pest control, many are safe to apply and unique in action and can be easily processed and used (Talukder and Howse, 1995).

In recent years much interest has been centered around the family *Meliaceae* to produce a number of terpenoids (Chatterjee and Kundu, 1967) of diverse structural skeleta which has been reported as toxic principles and have traditionally been used as fish or dart arrow poisons (Nishizawa *et al.*, 1984). This induced to examine *A. rohituka* W. & A. (syn= *Aphanamixis polystachya* Wall and Parker) commonly known as Pithraj/ Pitraj/ Tiktaraj/ Royna in Bengal. Its bark, leaves and fruits taste bitter and are widely used in folk medicine (Chatterjee *et al.*, 1970). The bark is used as an astringent (Watt, 1889), for spleen, liver, tumours, for abdominal diseases and in rheumatism (Agnihotri *et al.*, 1987b).

A. rohituka seeds are used to produce ghees/powders/bolmes/enemas to cure abdominal tumours (Hartwell, 1970). The seed oil is burnt by the poorer classes and is used as a stimulating liniment in rheumatism (Watt, 1889) and it is suitable for manufacture of soap (Razzaque *et al.*, 1983). The fruits after the removal of bitter oil are generally used as fertilizers. The oil, when burnt on small earthen plate with cotton wicks, are said to repel mosquitoes and flies. Very recently the plant has been studied and proved as a source of repellents, antifeedants, toxicants and protectants in storage against stored grain pests (Talukder and Howse, 1995).

Villagers in Bangladesh use the seed oil to protect flies from the wounds and rub the body with it to protect leeches and other problematic water organisms before getting down into water bodies in the rainy season. So, it is likely to happen that this plant can give suitable constituents to produce pesticidal properties for a safer application.

Some optimistic workers have already isolated some interesting compounds from different parts of this plant, but it was not attempted or never taken as a complete work to be done. So, it needs an assemblaged step to conduct various types of bioassays and to go though phytochemical analysis for further invention of more interesting compounds which can help in the development of desirable pesticides and can promote chemotaxonomic characteristics of this species.

As part of the above mentioned aim the present research work has been done to evaluate the different extracts on different strains (CR-1, FSS-II and CTC-12 strain) of the red flour beetle, *Tribolium castaneum* (Hbst.) (Coleoptera:Tenebrionidae).

1.1. Background information on the target plant

Careful selection of plant material is obviously very important in order to arrive at useful compounds for pesticidal or medicinal application in the shortest possible time. Random collection is one method, but it is more judicious to face the selection on certain criteria. By way of illustration, plants used in traditional medicine are more likely to provide pharmacologically active compounds (Huxtable, 1992).

Choice of plants according to chemotaxonomical consideration is another possibility. If, for example, a search for xanthones is being undertaken, it is advisable to start by investigating families which are known to contain this class of natural products- the *Gentianaceae*, the *Polygalaceae*, or the *Guttiferae* if prenylated xanthones are required. Aqueous plant extracts which foam copiously are giving indication of the presence of saponins. Plant parts or extracts which are dark red in colour may show the presence of tannins. Field observations can also be very important. If a brush or a tree shows no sign of being attacked by pests and has neither pieces eaten out of the leaves nor discolourations due to the presence of some foreign organism, there is a good chance

that same metabolites are present in it which act as insecticides or antimicrobial agents (Hostettmann *et al.*, 1995).

The family *Meliaceae*, comprising about 50 genera and 1400 species, forms a large botanical family of mostly pantropical distribution (Willis, 1966). Of all the plant families the *Meliaceae* is among the more useful to man, chiefly for its high quality timbers and for the ease with which some species can be grown in plantations. Almost confined to the tropics, its species nevertheless pervade them, and occur, conspicuously, in a variety of habitats from rain forest to mangrove swamps and semi-desert (Pennington and Styles, 1975).

With a few exceptions most of the genera currently recognized as meliaceous have been regarded as such since the time of their discovery. But there has been persistent disagreement as to the number of genera and their circumscription and as to the best way to accommodate them in tribes and subfamilies. Even the number of families has been uncertain from the time of Jussieu (1789) to that of Harms (1940). A still more detailed account of the *Meliaceae* was published in 1830 by Jussieu. This thorough and painstaking work remained in use for a very long time. Jussieu's (1830) classification which is based on a longer number of characters than any previous one, is a definite improvement.

During the first half of the nineteenth century, a number of botanical works, dealing with the flora of restricted geographical regions, were published, particularly for parts of Asia, in which genera of the *Meliaceae* were mentioned. The *Meliaceae* are well represented in India, and the first account for this country was by Roxburgh (cited in, Pennington and Styles, 1975). A list of plants published in 1814 in his *'Hortus Bengalensis'* included the names *Heynea, Walsura,* and *Andersonia. Andersonia* was

described and illustrated under the new name *Amoora* by Roxburgh himself, in his *'Plants of the coast of Coromandel'* published in the year 1820 (cited in, Pennington and Styles, 1975).

Hooker (1862), in the *'Genera Plantarum'* of Bentham and Hooker followed Candolle (1824) but differed from Jussieu (1830) in uniting the *Cedrelaceae* and *Meliaceae*. All authors since then have done the same. Hooker (1862) included thirty seven genera in the *Meliaceae*, and all of them are fully and accurately described. Some of the genera he recognized have since been split into two or more and others have been subordinated to synonymy, but the majority are still accepted today. These genera are arranged in tribes: Tribe I *Melieae,* contains *Quivisia, Calodryum* (=*Turraea*), *Turraea, Vavaea, Naregamia, Munronia, Melia,* (including *Azadirachta*), and *Mallea* (=*Cipadessa*); Tribe II *Trichilieae,* contains *Dysoxylum, Chisocheton, Epicharis* (=*Dysoxylum*), *Cabralea, Sandoricum, Aglaia, Milnea* (=*Aglaia*), *Lansium, Amoora* (=*Aglaia*), *Synoum, Guarea, Dasycoleum* (=*Chisocheton*), *Ekebergia, Walsura, Heynea, Beddomea* (=*Aglaia*) *Moschoxylon* (=*Trichilia*), *Odontandra* (*Trichilia*), *Trichilia, Owenia, Carapa* (including *Xylocarpus);* Tribe III *Swietenieae,* contains *Swietenia, Khaya, Soymida, Chukrasia* (Chickrassia), *Elutheria* (=*Schmardaea*); Tribe IV *Cedreleae,* contains *Cedrela, Chloroxylon* and *Flindersia.* In addition to a number of genera now regarded as synonyms, a considerable number have been described during the last one hundred years.

Candolle (1878), in *'Monographiae Phanerogamarum'*, resurrected *Vavaea* and *Walsura*, but reduced *Aphanamixis* to *Amoora.* Candolle's circumscription of *Dysoxylum* included *Didymocheton* and *Epicharis, Guarea* included *Ruagea, Carapa* included *Xylocarpus,* and *Cedrela* included *Toona.*

In 1896, Harms published his first account of the *Meliaceae* in Engler and Prantl's *'Die Natürlichen Pflangenfamilien'*. In this work he described 4 new genera and *Aphanamixis* was again reinstated as a genus in this work. It was also similar to those of Hooker (1862) and of Candolle (1878) but contains some important differences. He divided the *Meliaceae* into three sub-families and tribes were then six in number: I *Carapeae,* II *Turraeae,* III *Vaveae,* IV *Melieae,* V *Azadirachtieae* and VI *Trichilieae.* In the second edition of *"Die Natürlichen Pflanzenfamilien"*, Harms (1940) did not alter the circumscription of the sub-families, except to include those genera described since 1896. His division of the *Melioideae* into tribes remained virtually unchanged, except that *Azadirachta* was included with *Melia* in the tribe *Melieae* reducing the number of tribes into five.

This taxonomic perplexity was a lengthy phenomenon since it was a difficult job to differ meliaceous species each other and Pennington and Styles (1975) dealt with it for the later years. Most of the genera they recognized differ from all others in the family and at least two characters were common to all species. Consequently they considered that *Amoora* should be reduced to *Aglaia* and they proposed a new type of classification for the family *Meliaceae.*

Kostermans (1982c) disagreed with Pennington and Styles (1975) to include *Amoora* in *Aglaia.* He mentioned that the genus is so entirely different from *Aglaia* in its inflorescence and flower characters, and it seems better to keep it like as it was. He was working for the Family *Meliaceae* for long been time and reported about the title species *A. rohituka* citing his specimen number as Kostermans 28066, (Kostermans, 1982a).

Being a family member of the heavily studied Neem plant, *Azadirachta indica* A. Juss. and being considered as a medicinal plant in India and Bangladesh, *Amoora*

rohituka W. & A. is selected for this investigation. The title plant *A. rohituka* was in a clumsy of nomenclature because of some confusions about its proper botanical identity (Razzaque *et al.*, 1983). Mabberly in the year 1982 published on behalf of the *Malaysian Forester* giving a new information on the derivation of the generic name *Ricinocarpodendron* Boehmer. This generic name, validly published in 1760, and was considered to be referable to a tree with flowers of the rosaceous kind and was recognized as meliaceous one. A description and drawing (Fig. 1.) published by Amman (1736) is such that he must had seen the living plant or had a description thereof, for he mentions the colour of the flowers and of the fruits at different stages of development. Elsewhere, he described living plants from the St. Petersburg (=Leningrad) Botanic Garden and his herbarium was at Leningrad, but Mabberley (1982) assured that there was no specimen to be found there with his name *Ricinocarpodendron*.

Fig. 1. Amman's (1736) *Ricinocarpodendron* after Mebberley, (1982).

Fortunately, the published plate is clearly recognizable and represents the widespread Indomalayan genus, currently known as *Aphanamixis*, probably *A. polystachya* (Wall.) R.N. Parker. Although Amman's (1736) name *Ricinocarpodendron*, would seem rather inappropriate in some respects and might have been more usefully applied to say *Nephelium* (*Sapindaceae*), and it is the earliest name for *Aphanamixis*. This genus is small (about six species from China, India and Malaysia to the Solomon Islands) and only lately has its distinctness been argued cogently. In consequence, these plants, none of which is of any real commercial importance, have had many names in *Aglaia* Lour. and *Amoora* Roxb. while *Aphanamixis* has been used recently to induce *Sphaerosacme* Wall. and species now referred to *Reinwardtiodendron*. Mabberley (1982) then advised, therefore, that there is very little chance that an attempt to conserve *Aphanamixis* against *Ricinocarpodendron* would be successful, and proposed, rather reluctantly, that the latter name be taken up again.

Mabberley (1982) gave the key to the Malayan species of *Reinwardtiodendron* as follows: *Reinwardtiodendron palystachyum* (Wall.) Mabb. **comb. nova.** *Aglaia polystachya* Wall. in Roxb., Fl. Ind. **2**:429 (1824). Type: India, Assam and Silhet, '1821' *de Silva* in EIC 1277 (K-W! holo; K!). *Buchanania spicata* Roxb., MSS., *B. paniculata* Roxb., MSS. *Aphanamixis grandifolia* Bl., Bijdr.: 165 (1825). Type: Indonesia, Java, Mt. Salak, *Blume s. n.* (L! holo) *Guarea amaris* Buch. -Hom. in Mem. Wern. Soc. **6**:307 (1832). *Andersonia rohituka* (Roxb.) Fl. Ind. **2**:213 (1832). *Amoora rohituka* (Roxb.) W. & A., Prodr. **1**:119 (1834). *Aphanamixis rohituka* (Roxb.) Pierre. Fl. For Cochinch., Sub. t. 334 (1895) and t. 344 (1896). *A. polystachya* (Wall.) R.N. Parker in Ind. Flor. **57**: 486 (1931). This species is found throughout the range of the genus and has been described under many names in different parts thereof.

1.2. Morphological attributes, systematic position and distribution of the title species

Amoora Roxb. a genus of trees belonging to *Meliaceae* comprising some 15 species, inhabitants of the tropical and extra-tropical regions of Asia and Australia, 12 occurring in India and one being endemic to Australia (Watt, 1889). But (Anon, 1952) mentioned *Amoora* as a genus of evergreen trees, comprising about 25 species. The Generic name *Amoora* is derived from the Bengali vernacular name *Amúr*. The local names of the title species *Amoora rohituka* W. & A.: Pitraj/Pithraj/Pittiraj/Tiktaraj/Royna in Bangla; Harin hara/Harin khana in Hind; Sikru in Kol.; Sohaga in Oudh; Bandriphal in Nep.; Lota amari/Amora amari in Ass.; Okhioungza/Okhyang in Magh.; Shem maram (the red wood plant) in Tam.; Chaw-a-manu/Rohitakah in Tel.; Shem maram in Mal.; Rohituka in Sans.; Hingal gass in Singh.; Thitni/Chayan-ka-you in Burm.; (Watt, 1889).

The specific characteristics are as follows: leaves 1-3 feet long, leaflets 9-15, oblique, quite entire in size 3 to 9 by 1.33 to 4 inches; young parts tawny, closely pubescent, early glabrescent; inflorescence sub-dioecious, paniculate, female spicate or racemose; flowers white, bracteate, sub-sessile; male spikes panicled, female simple; calyx 5-partite, petals 3, anthers 6, ovary 3 celled with 2 superposed ovules in each cell; stigma sessile, style elongated, capsule subglobose, thickly coriaceous, 3-4 celled and seeded, seeds with a fleshy aril, hilum ventral (Watt.1889). Backer and Bakhuizen Van Den Brink, (1965) indicated in the *Flora of Java* that the leaves are spirally arranged, thinly or thickly coriaceous and trees hairy with stellate scales. The chromosome number of this title plant is n=18, 2n=76, C. 150 (Pennington and Styles, 1975).

Pending a complete revision of the genus, the transforms currently being prepared are mentioned here to give a nearly complete account of *Amoora* species in brief: *Reinwardtiodendron polystachyum* (Wall.) Mabb. **comb. nova.** *Amoora rohituka*

(Roxb.) W. & A. Type: India, Assam, Silhet; *R. Sumatranum* (Miq.) Mabb. **comb. nova.** *A. sumatrana* Miq. Type: Indonesia, Sumatra, near Padang; *R. borneense* (Miq.) Mabb. **comb. nova.** *A. borneensis* Miq. Type: Indonesia, Borneo, Mt. Prararawin; *R. cumingianum* (C.DC) Mabb. **comb. nova.** *A. cumingiana* C.DC, Type: Philippines; *Aglaia malaccensis* (Ridley) Panell, **comb. nova.** *Amoora malaccensis* Ridley. *A. ridleyi* (King) Pannell, **comb. nova.** *A. ridleyi* King. *A. rubenscens* (Hiern.) Pannell, **comb nova.** *A. rubescens* Hiern., *A. rubiginosa* (Hiern.) Pannell **comb. nova.** *A. rubiginosa* Hiern. Furthermore, *Amoora cucullata* Roxb., *Amoora decandra* Hiern and *Amoora spectabilis* Miq. or *Amoora wallichi* King., these three are the Indo-Himalayan species. New another species *A. beddomei* Kosterm. of Cylonese origin introduced by Kostermans (1982b).

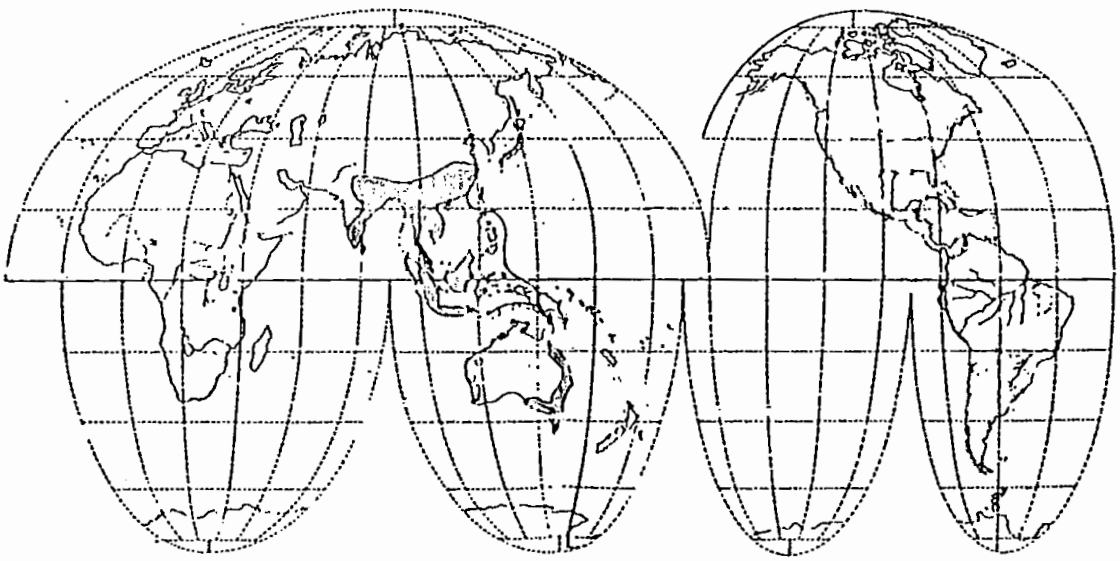


Fig. 2. The World map showing the distribution of the title species *A. rohituka*

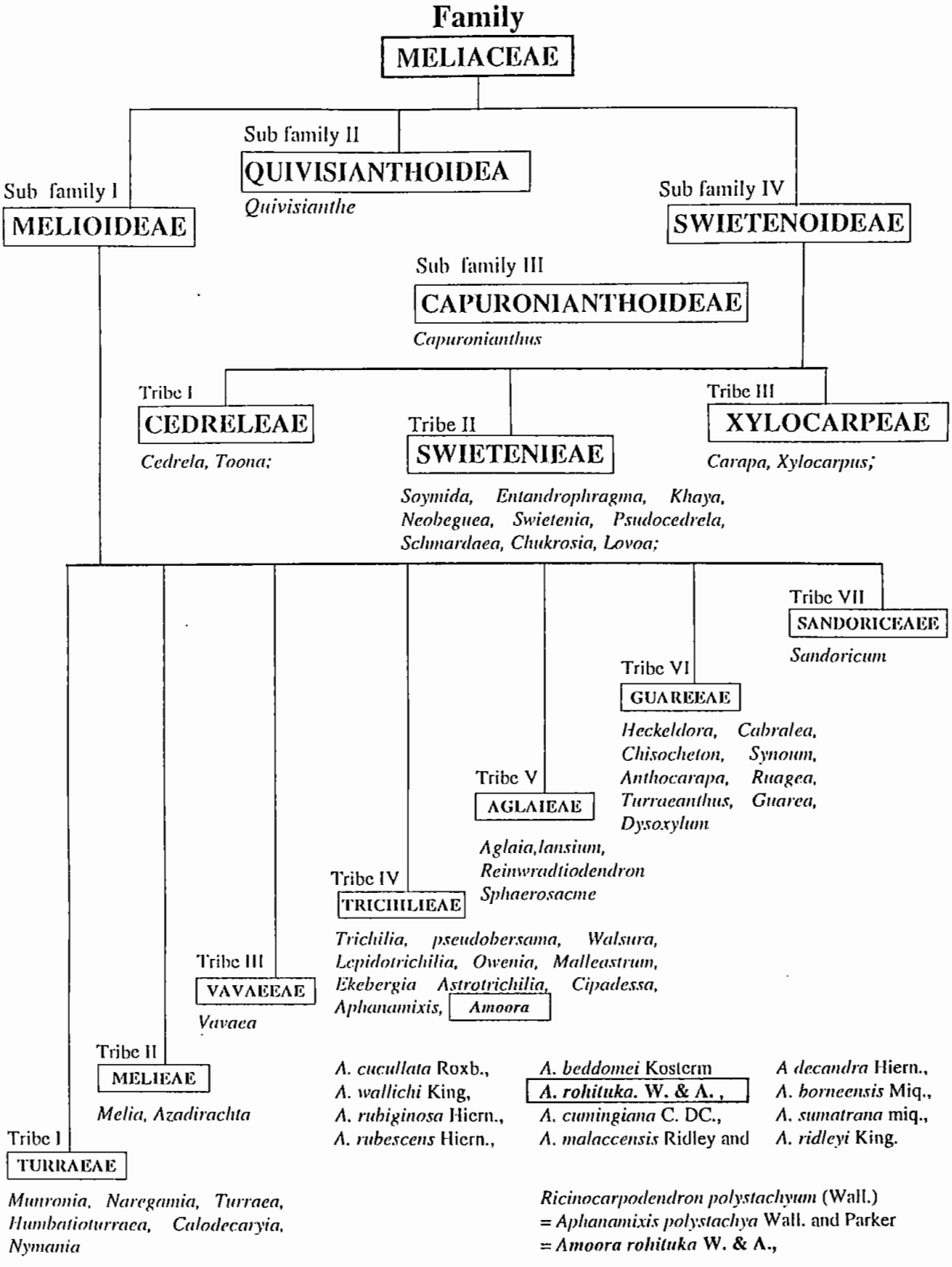


Fig. 3. Infra structure of the family *Meliaceae.*

***Amoora rohituka* W. & A.**
(Syn. *Aphanamixis polystachya* Wall & Parker) (Meliaceae).

1.3. Social utilities of the title plant

A. rohituka (*Meliaceae*) is a useful timber tree in Bengal. The trunk of this plant is hollowed out to make big canoes called 'Odis' in Nicobarese by the Nicobarese tribals of the Andaman and Nicobar Archipelago (Dagar, 1986). Its seeds, bark and leaves are employed in our indigenous system of Indian medicine (Chandrasekharan and Chakrabortty, 1968), as an astringent, for spleen, liver, tumours, for abdominal diseases and in rheumatism (Chopra *et al.*, 1956; Kirtikar and Basu, 1935).

In Bengal an oil is collected from the seed. The natives, where the tree grows plentifully, extract this oil, which they use for various economic purposes. The oil is used by the poorer classes as fuel for lamps and as a stimulating liniment in rheumatism. The seeds are fried and bruised, then boiled with water, when the oil floats on the top (Watt, 1889). The seeds are acrid with a sharp taste, refrigerant, laxative, anthelmintic, cure ulcers, diseases of the blood, of the eye and of the ear; lessen muscular pain as reported in the Ayurveda. Villagers in Bangladesh use the seed oil to protect flies from the wounds and rub the body with it to protect leeches and other problematic water organisms before getting down into water bodies in the rainy season.

The bark is used in spleen and in liver diseases, tumours and in abdominal complaints. The powdered bark is said to be very beneficial in cases of enlarged spleen. But, it was administered to a case of enlarged spleen with no benefit. It was later administered to cases of enlarged spleen and enlargement of liver of infants and the result was unsatisfactory (Kirtikar and Basu, 1935).

Ghees, powders, bolmes and enemas of seeds are used against abdominal tumours in India as reported in *Charaka Samhita* as indicated by Hartwell, (1970).

1.4. General components of the title plant and its related species

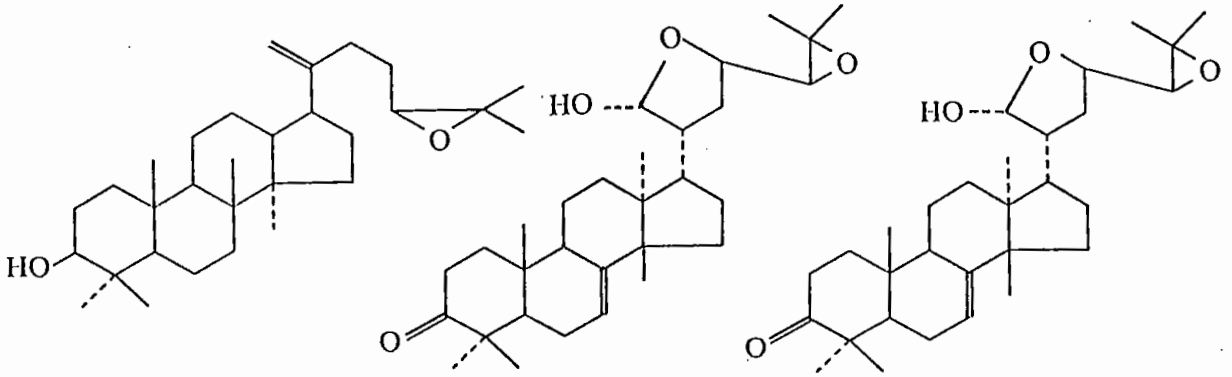
Plants of the family *Meliaceae* have repeatedly been the object of chemical study on account of the occurrence of interesting di- and triterpenoids (Chatterjee *et al.*, 1970) and of volatile oils (Hegnauer, 1969a,b). The presence of a large number of terpenoids of diverse structural skeleta in the family is well documented (Chatterjee and Kundu, 1967). The presence of alkaloids has been recorded in several species of *Meliaceae*. These plants grow luxuriantly in Bengal and their leaves and fruits taste extremely bitter because of the presence of the bitter substances which are collectively called 'meliacins'. These *Meliaceae* constituents, occurring either as esters or in the free state, are all chemically closely related and from the bitter principles of the meliacin group, are called 'limonoids' (Dreyer, 1968; Connolly *et al.*,1970).

During the intervening years after the constitution of limonin was established in 1960 by Arigoni *et al.*, a number of genera of this family have been investigated. The most extensively studied genera are *Khaya, Entandrophragma, Melia* and *Cedrela* (Banerji and Nigam, 1984), but most of those works were in the field of phytochemical analysis of new lead compounds which revealed the presence of terpenoids, limonoids, alkaloids, saponins and many different polyphenols. Limonin, the characteristic bitter principle of citrus species which are also found in the *Rutaceae* family was also reported. As is now known, a great number of compounds isolated from the same or a botanically related family *Meliaceae,* have a structure similar to limonin and comprise the bitter principles of the limonin group, (structure m, Chart-3) which are limonoids. It could be added that these bitter principles which have been isolated from the *Meliaceae* family have cytotoxic activity (Pettit *et al.*, 1983).

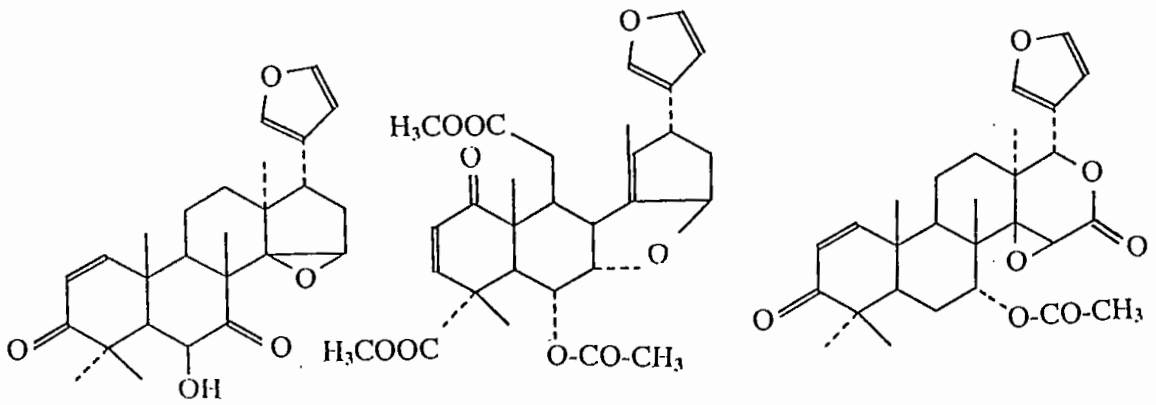
A number of *Meliaceae* speices are well known medicinal plants, such as *Amoora rohituka* (India), *Khaya ivorensis* (Nigeria), *Melia azadirachta* (Columbia), *Sandoricum indicum* (Mexico) and *Trichilia hirta* (Venezuela), have been used in the primitive treatment of cancer (Hartwell, 1970). A series of tetranortriterpenoids, as a source for

potentially useful antineoplastic agents have been uncovered which inhibit growth of the Murine P-388 lymphocytic leukemia (PS system) cell line of the National Cancer Institute (Pettit *et al.*, 1983).

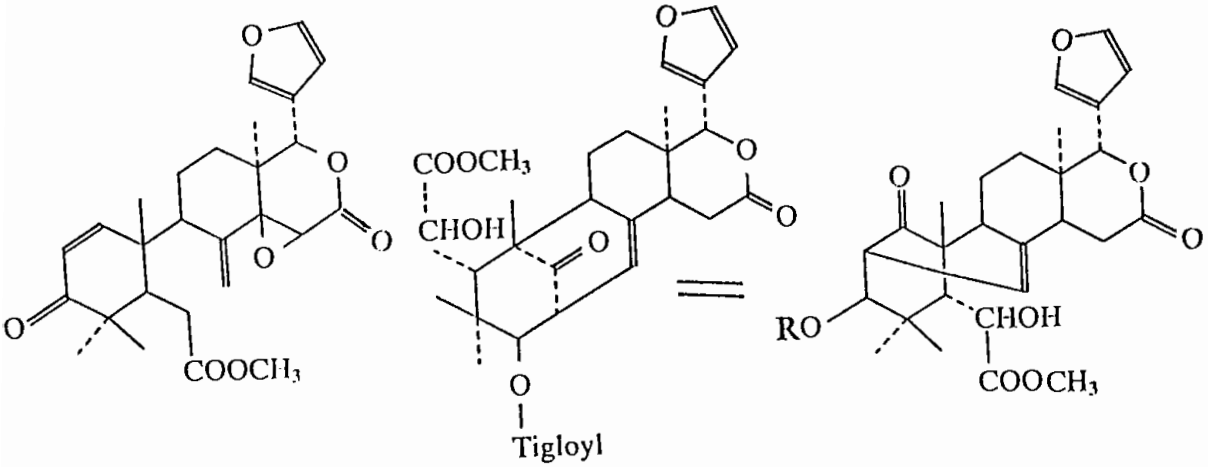
The characteristic terpenes of the family are Aglaiol, $C_{30}H_{50}O_2$; Melianon, $C_{30}H_{46}O_4$; Turraeanthin, $C_{32}H_{50}O_5$ etc., and the characteristic bitter principles are Cedrelon $C_{26}H_{30}O_5$; Nimbin, $C_{30}H_{36}O_9$; Gedunin, $C_{18}H_{34}O_7$; Andirobin, $C_{27}H_{32}O_7$ and Sweitenin, $C_{32}H_{42}O_9$. Drawn structures of these compounds are given in chart 1.



Aglaiol Melianon Turraeanthin



Cedrelon Nimbin Gedunin



Andirobin Swietenin

Chart 1. Characteristic constituents of the family of the title species.

These interesting compounds have some bio-active nature on different organisms. It is possible to control many human and animal pathogenic micro-organisms with antibiotics that are derived initially from either micro-organisms or plant secondary metabolites mentioned above. So, interests in native plants as potential sources of new antibiotics, anticancer agents and other pharmaceutical agents has been rewarded by the discovery of an unexpected diversity of new natural products (Sundarrao *et al.*, 1993).

A number of *Meliaceae* plants have been subjected to anticancer test given some surprising results in table 1.

MELIACEAE

Botanical Name	Common Name	Plant Part	Preparation	Type of disease	Comments	References
Aglaia roxburghiana (Wight & Arn.) Miq.	Priyanguka, Priyangu	-	ghees, powders, bolmes, enemas	Abdominal tumours	India	Kaviratna, 1888-1909
Aglaia roxburghiana (Wight & Arn.)	Priyangu	-	clarified butter	Abdominal tumours	India	Hoernle, 1893-1912
Amoora rohituka (Roxb.) Wight & Arn	Rohita creeper	seed	ghees, powders, bolmes, enemas	Abdominal tumours	India	Kaviratna, 1888-1909
	-	wood	-	Tumours	Cochin-China	Loureiro, 1790
Dysoxylum leuretri Pierre	Marinheiro de folha					
Guarea spiciflora A. Juss.	harga	root-bark	-	Skin	Brazil	Martius, 1843
	Trompillo	leaf	with coconut oil	indurations	Venezuela, native medicine.	Pompa, 1875
Guarea trichiloides L.				Tumours		
	African or Lagos	bark	lotion		Nigeria	Ainslie, 1937
Khaya ivorensis A. Chev.	mahogany			Tumours		
Melia azadirachta L.	Nimva	-	ghees, powders, bolmes, enemas	Abdominal tumours	India	Kaviratna, 1888-1909
Melia azadirachta L.	Nim, margosa	leaf	Poultices	Tumours	India	O'Shaughnessy, 1841
Melia azadirachta L. (*A. indica*)	Neem-tree	leaf	poultices	Glandular tumours	India	Drury, 1858
Azadirachta indica)	Margosa (Neem)	oil	injections	Parotid tumours and epidermoid carcinoma	India, report of two patients	Chatterjee, 1961
Melia azadirach L.	Paraiso	leaf	-	Tumours	Colombia	Arbeláez, 1956
Melia azadirach L	-	root	-	Tumours	India, (Ayurvedic medicine)	Chopra, 1933
Plaeroxylon obliquum Radlk.	-	Juice of wood	-	Warts	South Africa, (used by the Africans).	Watt and Breyer-Brandwijk, 1962
Plaeroxylon utile Eckl. and Zeyh.	Um-tote, Sneeze-wood	twig juice	painted on	Warts	Bantu folklore.	Hewat, 1908
Sandoricum indicum Cav.	-	root, leaf	-	Tumours	-	Kosteletzky, 1831-1836
Sandoricum indicum Cav.	-	leaf	-	Tumours	-	Rosenthal, 1861
Swietenia sp.	Tzopilodtzonte-comatl	-	-	Tumours	Mexico, 1577	Hernández, 1942-1946
Swietenia sp.	Tzopilotzontecomatl, tzopilotl	seed	oily liquid	Tumours	Mexico	Ximenez, 1888
Toona ciliata Roemer (*Cedrela toona* Roxb. ex Rottl. & Willd.)	Tun, toon	bark, leaf	liniment	Indurations of the spleen	Acc. to Rumphius used by the Javanese.	Fleming, 1810
Trichilia hirta L.	Trompillo, cazabito	leaf	with coconut oil	Tumours	Venezuela, native medicine.	Pompa, 1875

Table 1. A checklist of the *Meliaceae* activities against cancer.

Amongst all meliaceous plants *A. rohituka* is well known and widely used in folk medicine. A systematic chemical investigation of the leaves, seeds, fruit-shell, barks and roots yielded a number of such interesting compounds, viz. amoorinin, rohitukin, rohitukine, aphanamixin, etc. Interesting alkaloids, limonoids, diterpenes, triterpenes, tetranortriterpenoids, glycosides and even saponins were also there in this title species.

Having all possible application of most of the important species of the family *Meliaceae,* all over the world, another pharmacological interest has created among the scientists since the given species has been used against cancer in the past in India by the native people.

The plant was studied a little mostly for chemotaxonomical purposes, and most of the previous workers were chemists. But some were interested in biological context to have toxicological or pharmacological data and carried out some works in this regard, of which a few works done by bioassay guided fractionation and this induced to examine the plant to find more bio-active constituents from different parts of this plant and to isolate some of its interesting compounds to enhance the expedition of development of natural means to protect pests and to cure diseases and to develop chemotaxonomic progress as well.

1.5. Constituents reported from the target plant

The species *A. rohituka* has been studied and a very few compounds are isolated without indicating their activities in different aspects of pesticidal, medical and pharmacological interests. Different parts of this plant, viz. leaves, seeds, fruit-shell, stem-bark and roots were subjected to afford a number of compounds within passed few decades. A diterpene alcohol (Aphanamixol), an alkaloid (rohitukine), a prieurianin-type limonoid (rohituka-7), two limonoids (amoorinin and rohitukin or 3-di-hydroandirobin), a triterpene (aphanamixin), a *seco-ring* β-tetranortriterpenoid (aphanamixinin), three glycosides (1,5-dihydroxy-6, 7, 8- trimethoxy-2- methyl -anthraquinone -3 -*O* -β -D-xylopyranoside, naringenin 7, 4′-dimethyl ether -5-*O*-α-L-rhamnopyranoside and (24 R)-24-ethylcholestra-5, 22-diene-3-*O*-rhamnoside, a β-sitosterol and a stigmasterol, an aglaiol-3-*O*-rhamnosyl-xyloside, , three saponins (poriferasterol-3-rhamnoside, betulin-3-β-*O*-β-D-xylopyranoside, and stigmasta-5, 24 (28)-dien-3β-*O*-β-Dglucopyranosyl-*O*-α-L-rhamnopyranoside) and a flavone glycoside (8-C-methyl-quercetin-3-*O*-β-D-xylopyranoside) have been reported by previous workers. These findings were not received with their complete stereochemistry and pharmacological or pharmacognostical information, rather most of them were isolated without activity guided fractionation. Only aphanamixin was reported with its strong bio-active action, thus information on toxicological affair is available, and this is considered as the major toxic constituent of the title plant. The alkaloid found in this species is declared as the first alkaloid of the family *Meliaceae*. A complete checklist in this regard is given in tables 2a and 2b, and the structures given in chart 2.

Plant part	Molecular formula	Mass	Melting point	Name	Activity (Authors)
Leaves	$C_{20}H_{36}O_2$	M^+308	130°C	Aphanamixol (a diterpene alcohol)	Neutral* (Chandrasekharan and Chakrabortty, 1968)
	$C_{16}H_{19}NO_5$	M^+305.1264	218-219°C	Rohitukine (an alkaloid)	nr (Harmon *et al.*, 1979)
Seeds	$C_{34}H_{12}O_{13}$	-	275-280°C	Rohitukin (a limonoid)	(Connolly *et al.*, 1976) antifeedant against tobacco budworm, (Lidert *et al.*, 1985); P-388:ED_{50}=100 (Pettit *et al.*, (1983);
	-	-	-	Rohituka-7 (prieurianin-type limonoid)	antifeedant against tobacco budworm, (Lidert *et al.*, 1985)
	-		65-66°C	Stigmasta-5, 24 (28) -dien 3β-*O*-β-D glucopyranosyl-*O*-α-L-rhamnopyranoside (a saponin)	nr (Bhatt *et al.*, 1981)
Fruit-pericarp	$C_{32}H_{50}O_5$	M^+514	232-234°C	Aphamamixin (triterpene)	(Chatterjee and Kundu, 1967); (Chatterjee *et al.*, 1970); The major toxic constituent, LC_{50} 0.06 ppm (48h) (Nishizawa *et al.*, 1984)
Stem-bark	$C_{27}H_{34}O_7$	M^+470	210°C	Aphanamixinin, (a *seco-ing* β-tetranortriterpenoid)	nr (Chandrasekharan *et al.*, 1968); (Chatterjee *et al.*, 1970)
	$C_{23}H_{24}O_{12}$	-	158-162°C	1.5-dihydroxy-6,7,8-trimethoxy -2-methyl-anthraquinone-3-*O*-β-D-xylopyranoside.	nr (Srivastava and Agnihotri 1985)

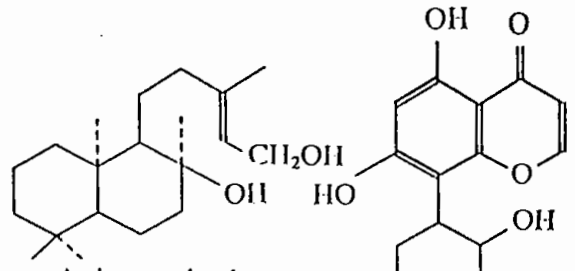
nr = Not reported, * = Found active on *Candida albicans* and *Bacillus subtilis* in the present investigation.

Table 2a. A checklist of *Amoora rohituka* properties.

Plant part	Molecular formula	Mass	Melting point	Name	Activity Authors
Stem-bark	$C_{23}H_{26}O_9$	-	125-128°C	Naringenin 7, 4′-dimethyl ether - 5-*O*-α-L-rhamnopyranoside	nr (Srivastava and Agnihotri,1985)
	$C_{27}H_{34}O_7$	M^+470	172-174°C	Amoorinin (a limonoid) (3-di-hydroandirobin)	nr (Agnihotri *et al.*, 1987a, 1987b)
	$C_{41}H_{68}O_{10}$	-	138-40°C	A Saponin	nr (Srivastava and Agnihotri, 1984)
	-	-	-	(24 R)-24-ethylcholestra-5, 22-diene-3-*O*-rhamnoside	nr (Srivastava and Agnihotri, 1985)
	-	-	-	β-sitosterol	Neutral (Srivastava and Agnihotri, 1984, 1985)
	-	-	-	Stigmasterol	nr (Srivastava and Agnihotri, 1984, 1985)
	-	-	-	Aglaiol-3-*O*-rhamnosyl-xyloside	nr (Srivastava and Agnihotri, 1985)
	$C_{35}H_{58}O_5$	-	122-125°C	Poriferasterol-3-rhamnoside (a saponin)	nr (Agnihotri, 1987)
Root	$C_{35}H_{58}O_6$	-	360°C	Betulin-3-β-*O*-β-D-xylopyranoside (a saponin)	nr (Jain and Srivastava, 1984)
	$C_{21}H_{20}O_{11}$	M^+448	-	8-C-methyl-Quercetin -3-*O*-β-D-xylopyranoside (a flavone glycoside)	nr (Jain and Srivastava, 1985)

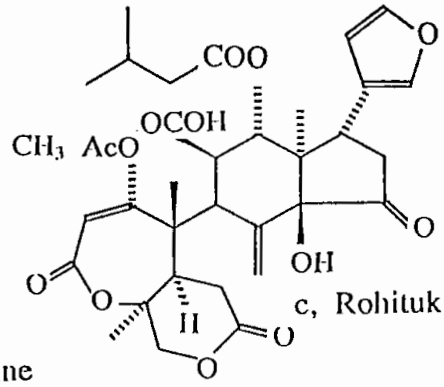
nr = Not reported, * = Found active on *Candida albicans* and *Bacillus subtilis*.

Table 2b. A checklist of *Amoora rohituka* properties.

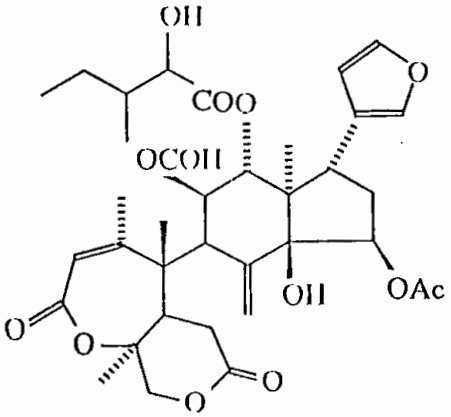


a, Aphanamixol

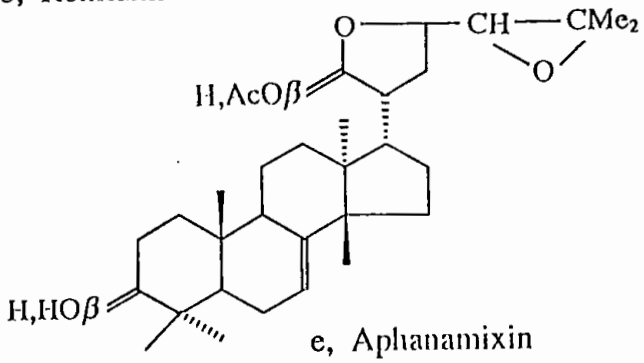
b, Rohitukine



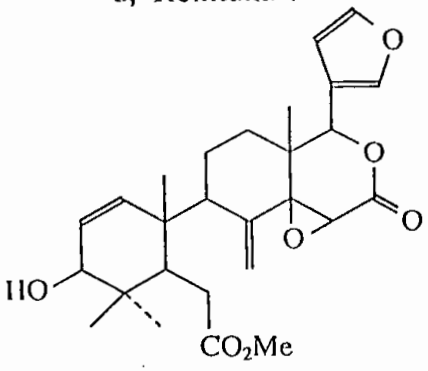
c, Rohitukin



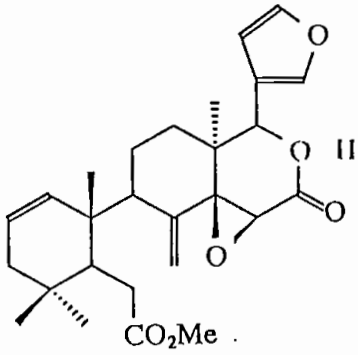
d, Rohituka-7



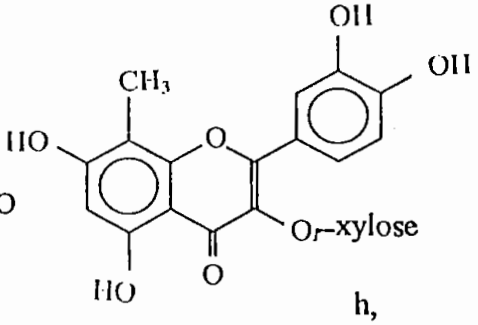
e, Aphanamixin



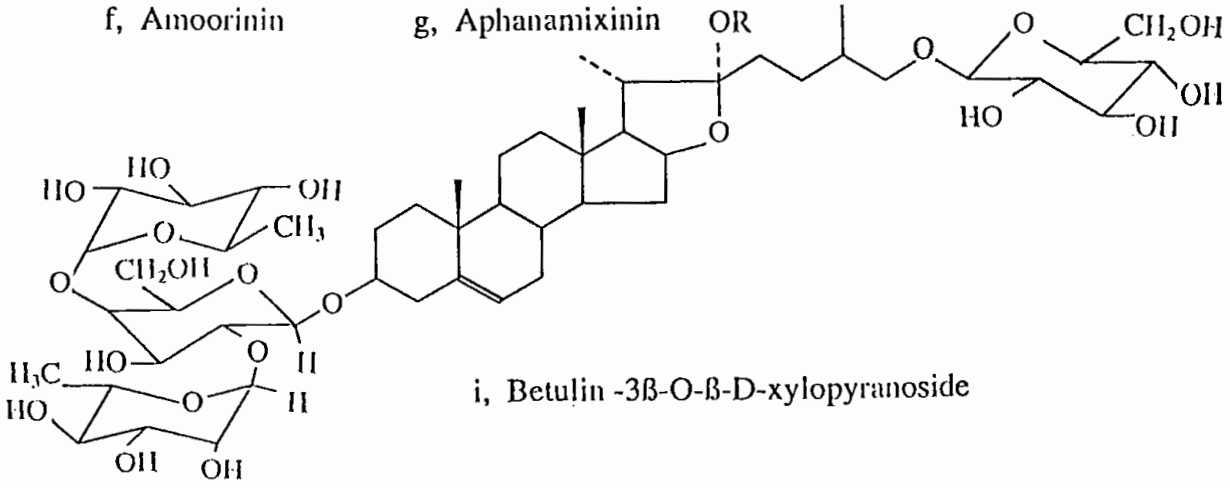
f, Amoorinin



g, Aphanamixinin



h,



i, Betulin -3ß-O-ß-D-xylopyranoside

Chart 2. Chemical structure of the constituents reported from the target plant

1.6. Bio-active and pharmacologically important principles reported from the given species

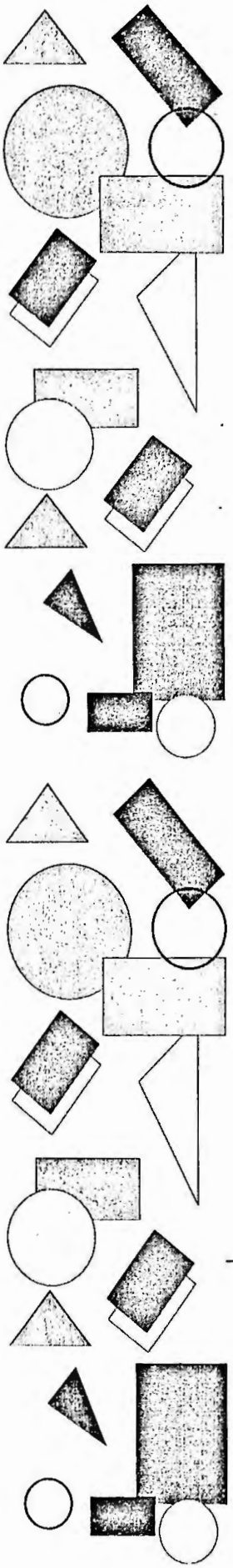
Amongst the phytochemists dealing with isolation, purification and structure elucidation of plant derived compounds some were involved in evaluating biologically active principles. Workers active with *A. rohituka* plant found a mentionable number of limonoids, saponins, glycosides, steroids and terpenoids of which a few are isolated by activity guided fractionation, and a very few of them were found active.

The major toxic component was a known triterpenoid, **aphanamixin** (structure e, Chart-2), derived from the petrol extract of the fruit-shell of *Aphanamixis polystachya* Wall. (Parker) (Chatterjee *et al.,* 1970). The fractionation was always monitored by toxicity against fish *Oryzias latipes*, and it showed toxicity of LC_{50} 0.06 ppm (48h), (Nishizawa *et al.,* 1984).

Rohitukin (structure c, Chart-2), was isolated from *A. rohituka* seeds, and found active against Murine P-388 lymphocytic leukemia cell line of the National Cancer Institute and the toxicity was ED_{50}= 100 T/C inactive (2→0.25mg) (Pettit *et al.,* 1983), and also found antifeedant against tobacco budworm (Lidert *et al.,* 1985).

Another limonoid **rohituka-7** (structure d, Chart-2), was also isolated from the seeds of the title plant showed activity as an insect-antifeedant while test was administered against Mexican bean beetle and southern army worm (Lidert *et al.,* 1985).

The major toxic constituent aphanamixin of the target species *A. rohituka* was also reported from the fruit-shell of the closely related species *A. grandifolia* (Chatterjee et al., 1970).



Chapter 2

Materials & Methods

Chapter 2 Materials and Methods

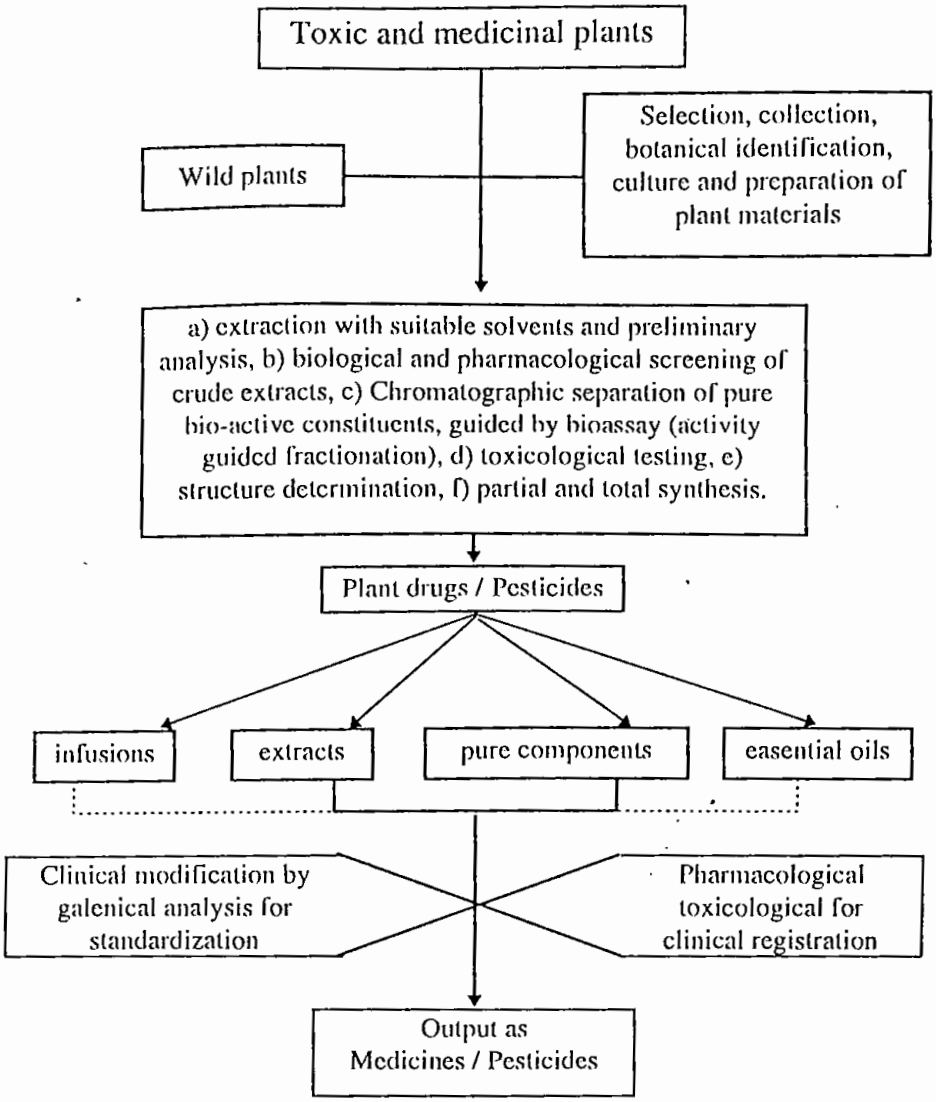
2.1. Investigation for further bio-active compounds from the title plant

Post harvest application of plant parts in the tropical and subtropical countries are still prevalent even in these days of development to protect stored grain pests. But the technique used is not scientific and it hampers conservation of same endangered species of plants. It needs more materials to make sure the storage free from pest infestation. By isolation of active principles from these plants, it could be possible to reduce the volume of utilization of plant materials, moreover, the rapid disappearance of tropical forests and other important areas of vegetation has meant that it is essential to have access to methods which lead to the rapid isolation and identification of bio-active natural products for this purpose.

Furthermore, we can control many human, animal and plant pathogenic organisms with antibiotics that are derived initially from either micro-organisms or plant secondary metabolites. Interest in native plants as potential source of new antibiotics, anticancer agents, pesticides and other pharmaceutical agents has been rewarded by the discovery of

an unexpected diversity of new natural products. Since plants may contain hundreds or even thousands of metabolites, this is currently a resurgence of interest in the vegetable kingdom as a possible source of new lead compounds for introduction into screening programmes. After development of multimedia techniques natural resources have been focused to be the potential sources for safe, biodegradable and more beneficial drugs, remedies or pesticides for a sustainable environment on the planet. Insects, mites, sponges, algae or even micro-organisms have also been subjected to yield active compounds in this regard, but plants are the most suitable source for such a propagation in the field of pesticide technology while some plants in different parts of the world are considered toxic and some are used in the traditional medicine. A literature search on the title plant offered some essential openings that this species bears repellent, antifeedant and toxicological properties which is subjected to go through screening and then isolation, purification and structure elucidation of essential constituents to develop natural non-hazardous biodegradable pesticides.

The approach adopted to obtain an exploitable pure plant constituent involves interdisciplinary work in botany, zoology, pharmacognosy, pharmacology, chemistry and toxicology as described by Hostettmann *et al.*, (1995) and can be shown in Fig. 5:



To simplify the technique of the pathway from the plant to the bio-active constituents (after Hostettmann *et al.*, 1995) could be shown as a diagram given below:

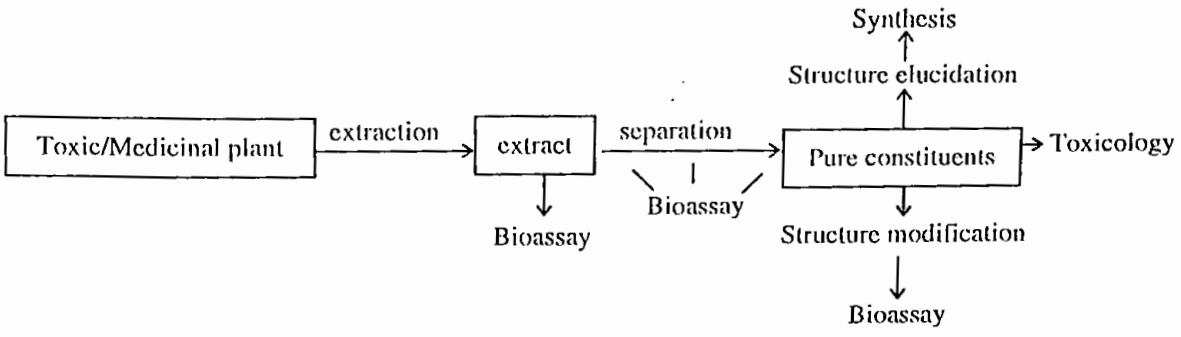


Fig. 5. The basic pathway from the plant to the bio-active constituents.

2.1.1. Selection of plant materials

In order to arrive at useful compounds in the shortest possible time, careful selection of plant material is obviously very important. Random collection is one method but it is more judicious to base the selection on certain criteria. By way of illustration, plants used in traditional medicine are more likely to provide pharmacologically active compounds (Huxtable, 1992), similarly folk use of toxic plants could be taken with desirable output.

In this investigation different parts of *A. rohituka,* viz. leaves, seeds, fruit-pericarp, stem-bark and roots have been collected for the presence of toxic, as well as, bio-active constituents since the plant is well known as a medicinal plant and also considered to contain toxic constituents. In case of very small plants, such as herbs, shrubs, grass, etc. normally the whole plant is subjected for extraction, because the distribution of constituents generally not vary too much. Being a large timber plant, the distribution of compounds in different parts of this plant is obviously different. The presence of constituents in the heart-wood may disappear in the leaves; similarly constituents in the roots may not be the same that present there in the fruits.

2.1.2. Selection of test insects

To test for insecticidal properties of the extractives of different parts of the title species *A. rohituka,* a test insect *Tribolium castaneum* (Hbst.) was selected, because it is a easy cultivable and noble laboratory insect has been used almost all over the world as a test insect for the tests of stored-grain pests. For a precise result three different strains have been taken in this investigation.

2.1.3. Chemical extraction of the collected materials

There are basically two methods for extracting compounds from plant materials. Which one to choose, depends on whether the aim is to extract the more polar compounds (especially glycosides) which are present in the cell vacuole, or to obtain the less polar aglycones present on the surface of the plant, in aerial parts, heartwood or roots. In the present study four solvents were selected to extract five different parts of *A. rohituka* separately. The ground dried material, viz. leaves 113.5g, seeds 100.84g, fruit-pericarp 100g, stem-bark 152.39g and 111g of roots were extracted with sufficient amount of petroleum spirit (Pet. spt.), ethyl acetate (EtOAc), acetone and methanol (MeOH) for each of the items successively. Separate extracts have been collected by the cool method after 72 hours of plunging for each of the material in each solvent separately. Extracts, thus, obtained are filtered and concentrated on a rotary evaporator at 40°C and only as residue is left and kept in a refrigerator after labelling.

For each item four solvents used, one after another, and the amount of materials were recorded deducing the amount of the extract afforded in the previous extraction for some conveniences in preparing doses for the insecticidal tests. The pathway for the extraction, in detail, used in this investigation is given in Fig. 6:

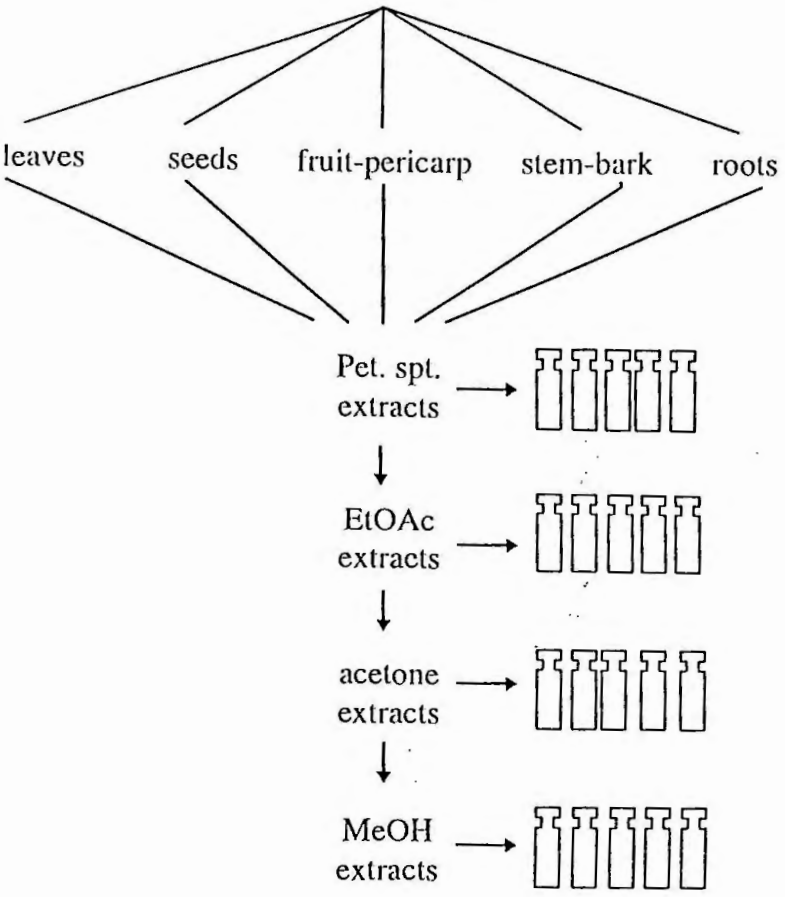


Fig. 6. Collection of extracts in different solvents from the plant materials.

2.2. Bioassays for active principles

Crucial to any investigation of plants with biological activities is the availability of suitable bioassays for monitoring the required effects. In order to cope with the number of extracts the capacity for high sample throughput is necessary. The test systems should ideally be simple, rapid, reproducible, and inexpensive. If active principles are only present at low concentration in the crude extract the bioassay is to be high enough sensitive for their detection. Another factor of special relevance to plant extracts is the solubility of the sample. Finding a suitable system can pose problems.

For the selection of bioassays to employ in research on plant constituents, the first step is to choose suitable target organisms (e.g. micro-organisms, insects, molluscs), isolated subcellular systems (enzymes, receptors, or organelles), cultured cells of human or animal origin, isolated organs of vertebrates, or whole animals. However, the right target has to be found for the right disease. The complexity of the bioassay has to be designed as a function of the facilities and resources available. A selection of these bioassays is shown in the table 3:

Activity	Target organisms
Insecticidal	1. *Tribolium castaneum* (Stored grain pest in the tropics)
Larvicidal	2. *Aedes aegypti* (Mosquito vector of yellow fever and Dengue)
Molluscicidal	3. *Biomphalaria glabrata* (Schistosomiasis transmitting snails)
Antifungal	4. *Cladosporium cucumerinum* (plant pathogenic fungus)
	5. *Candida albicans* (Human pathogenic yeast)
Antibacterial	6. *Bacillus subtilis* (Human pathogenic bacteria)

Table 3. List of test agents used in this investigation.

The list covers a variety of targets, ranging from a test for general toxicity with mosquito to snail killing activity. Different properties and types of ailment, including microbial afflictions and parasitic diseases, can be investigated in this way. Assays for antimicrobial agents occupy an important place in any screening programme. With the problems of drug resistance and limited spectrum of activity, new lead compounds are actively being sought (Mitscher and Rao, 1984).

2.2.1. Insecticidal test

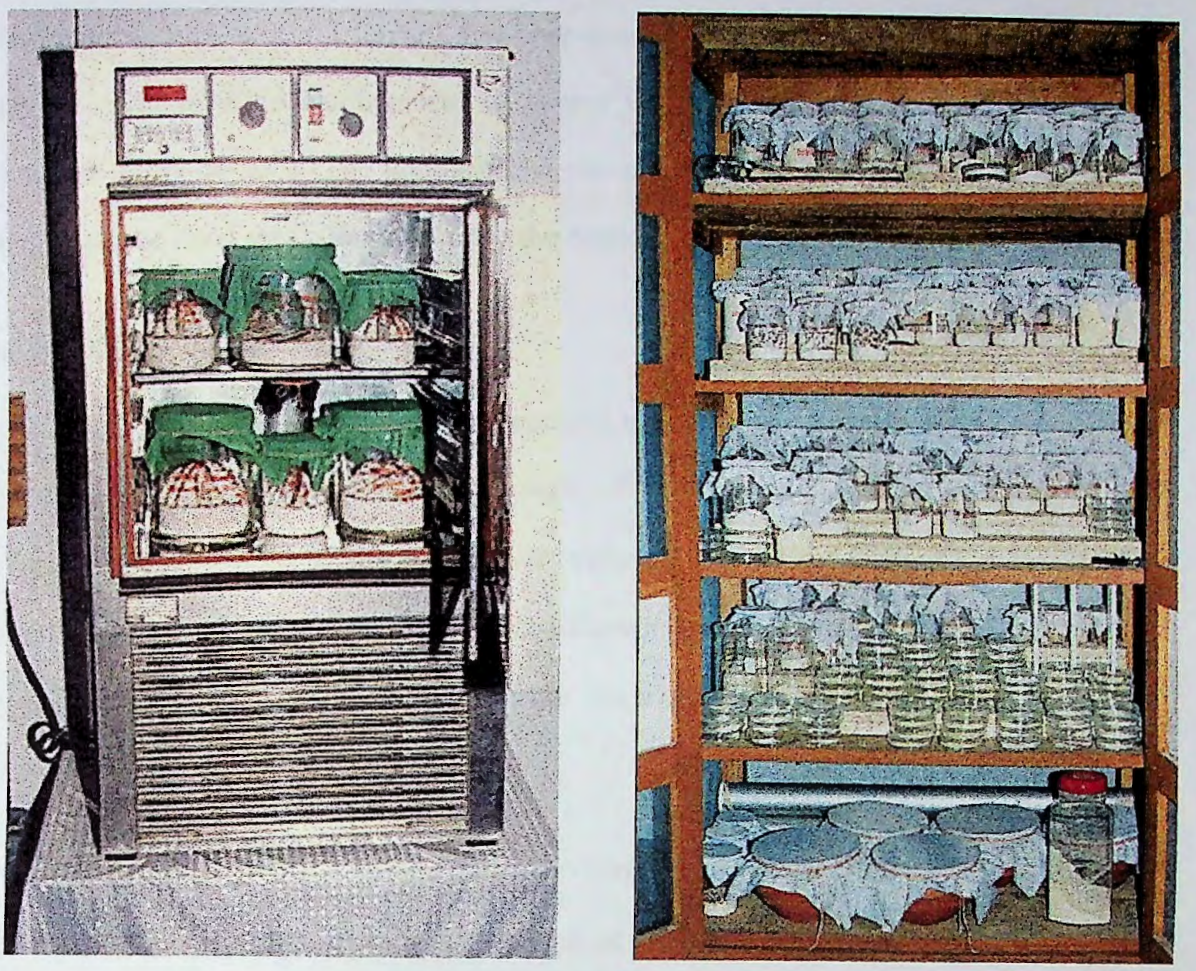
Biological test is an essential tool to evaluate activities of any compound that could be used to develop pesticides and medicines. Now-a-days, plant derived compounds are being subjected to produce insecticides or insect antifeedants or insect repellents. To conduct insecticidal tests of the materials it needs a easy cultivable test insect and a suitable way of investigation by bioassay with which it could afford all expected parameters we need for the summary conception.

Collection and culture of *T. castaneum* strains

Three standard strains of *T. castaneum* (Hbst.), i.e. CR-1, FSS-II and CTC-12 were collected from the Crop Protection Laboratory, Department of Agricultural and Environmental Science, University of Newcastle Upon Tyne, England; during September 1989. The strains were reared in the Crop Protection and Toxicology Laboratory, Institute of Biological Sciences, Rajshahi University, Bangladesh and cultures were made for the present study.

Preparation of the food medium

A Standard mixture of whole wheat flour with powdered dry yeast (19:1) was used as a food medium throughout the experimental period (Park and Frank, 1948; Park 1962; Zyromska-Rudzka, 1966). The cultures were maintained in glass beakers (500 ml) and pieces of filter papers were placed inside the beaker for the easy movement of the adult beetles. The beakers were covered with pieces of cloths tied with rubber band and kept in an incubator at 30°C ± 0.5°C without light and humidity control. For continuous and huge supply of the beetles mass cultures were maintained on a culture rack without temperature control.



With temperature control

Without temperature control

Fig. 7. Culture of test insects in the laboratory.

Collection of newly formed adults

Before starting experiments a huge number of beetles were reared to get a regular supply of the newly formed adults with approximately same age. For this 6 rearing media were prepared in large glass jars (120×150 mm) each of which contained 500 g of wheat flour properly mixed with required amount of yeast powder and 100 pairs of (male and female) adult insect were released therein. In regular intervals the cultures were

checked and the eggs were separated in sub-cultures sieving the medium in sieves (mesh 60 for eggs and mesh 30 for larvae). For dose-mortality experiment adults were separeted from the food medium using pieces of papers inside the culture beakers to allow the beetles to crawl on to it. The adults in the sub-cultures were collected by a camel hair brush from the food medium and used in the experiments.

Preparation and application of doses

For the preparation of doses the extracts were dissolved in with a definite amount of the solvent of extraction (the dry-weight of the dust subjected to extract with the solvent) was converted in the volumetric weight of the solvent at 20°C and this was considered as the highest dose, which was diluted step by step in a serial dilution on the basis of the range of activities found in the test experiments. The schematic pathway is given in the table 4.

The application of doses were carried out by topical treatment system. One µl of each of the doses were applied on the back of each insect with a micro-syringe. Forty insects were used for each dose with four replications on a 90 mm petridish separated each 10 of them by 30 mm rings and covered during the whole observation period. One control batch was also placed and all sets kept in an incubator at 30 ± 0.5°C. The mortality of the insects were recorded after 24-, 48- and 72- hours of treatment. The recorded mortality was then subjected to probit analysis according to Finney (1947) and Busvine (1971).

1. Source material:
   (Collected from:)
2. Solvent used:
3. Conversion constant of the solvent (C): gm.
4. Weight of the source material (W) = gm.
5. Weight of extract (E) = gm.
6. Functional weight (F)*: (W - E) = gm.
7. Converted amount (Ca): (F / C) = ml.

First dilution: $A = \frac{F / C}{E} =$ µl/µg.

Dose concentration $Dc = \frac{E \times 1000}{Ca} =$ µg/µl.

* The functional weight at any step will be treated as W in the next.

Table 4. Steps taken for the preparation of doses.

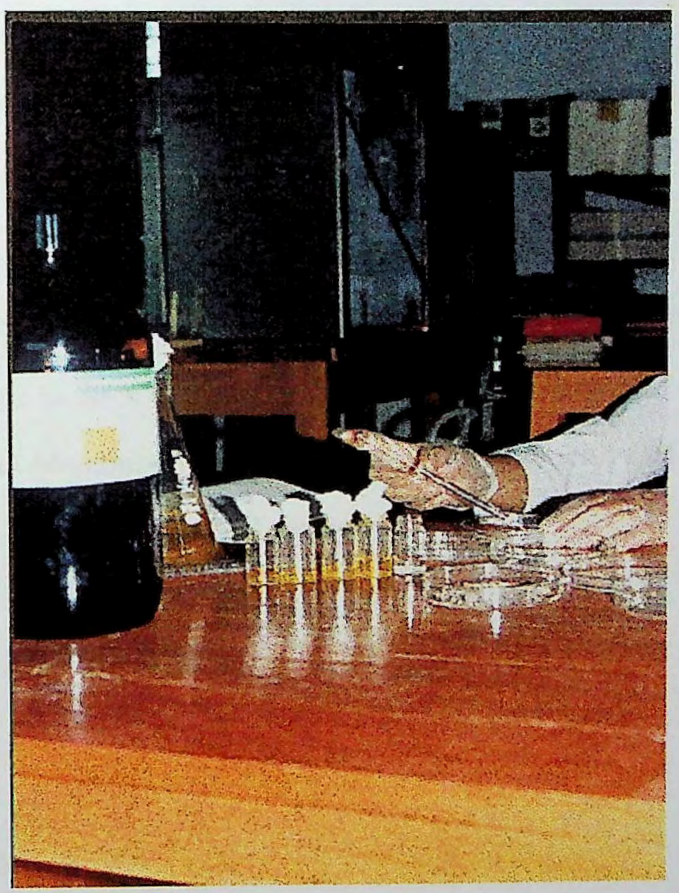


Fig. 8. Application of doses for insecticidal test.

2.2.2. Larvicidal test

A test for general toxicity has been done by a larvicidal test using the larvae of the mosquito vector of yellow fever and Dengue, *Aedes aegypti*. The eggs were supplied by Ciba Geigy Ltd. spawned on filter papers.

A piece of egg-bearing paper put into a beaker with fresh water (collected from the nearby stream 'La Sorge' followed over the University campus of the Université de Lausanne, Switzerland.) and kept in an incubator at 30° ± 0.5°C temperature for 24 h to afford sufficient number of larvae for the experiment.

For each sample 5.5 mg of each of the crude extract of different parts of the target plant *A. rohituka* taken in small tubes and added 110 µl of DMSO (for apolar extracts, viz. Pet. spt., EtOAc and acetone extracts) and H_2O for MeOH extracts and dissolved by using ultrasound bath or by a sonnicator tip. Up to half of each 10 ml tubes filled with fresh water 30 larvae were released in each and 100 µl of the dissolved extracts were added in different ones and mixed on vortex before adding more half (5 ml) stream water into each of the tubes to give a concentration of 500 ppm = 500 µg/ml. The tubes then placed in an incubator at 30 ± 0.5°C and mortality of the larvae were observed after 30 min. and 12-, 24- and 36- hours respectively. A control only with DMSO in fresh water also maintained during the experimental period.

2.2.3. Molluscicidal test

To test biological activities of any test sample, test on aquatic organisms could be a suitable manner. Schistosomiasis transmitting snails *Biomphalaria glabrata* were used for molluscicidal test of *A. rohituka* extracts.

Culture of Snails

An adaptation of the WHO method (*WHO Tech. Rep. Ser.* 1965) was performed along with the help of the system applied by Sukumaran *et al.*, (1994) and Zani *et al.*, (1993), who followed the procedure developed by Hostettmann *et al.*, (1982). *B. glabrata* snails were reared in aquaria with a continuous circulation of dechlorinated water maintained at 28°C through a filter system. Snails of uniform size (diameter of shell ~75 mm) were used.

Preparation and application of doses

Forty mg of each sample extract was dissolved in 100 ml of dechlorinated water to afford a concentration of 400 ppm. In cases, where the compound was not soluble directly in water it was dissolved in a small amount of EtOH that was then made up to a volume with dechlorinated water to produce a 1% EtOH solution. Control group of snails were placed in dechlorinated water or in a 1% EtOH solution in this case.

The snails were kept at 20°C for 24 h and then taken out from the small tanks and placed on a petridish. Light was shone from the bottom of the dish, and each snail examined for the presence of heart beat. Snails showing no heart beat were placed for 24 h in dechlorinated water and then reexamined to check mortality or recovery, the later being demonstrated by observance of heart beat. For active extracts the assays were repeated in duplicate at a lower sample amount to produce a concentration of 200 ppm or even 100 ppm of the extract.

2.2.4. Fungicidal test

The increasing incidence of opportunistic systemic mycoses in man and the associated therapeutic difficulties require the search for new antifungal drugs. Plant derived compounds may offer potential leads for novel agents against systemic fungal diseases, as demonstrated by Hufford and Clark (1988). As par of our continuing search for new bio-active constituents from *A. rohituka* 20 crude extracts of different parts of the plant in different solvent have been screened for antifungal activity against the plant pathogenic fungus *Cladosporium cucumerinum* and the human pathogenic yeast *Candida albicans*. These two fungi are suitable indicator organisms for screening purposes. Bio-autographic assays on TLC with these two micro-organisms can be used for evaluation of the activity of chemically applied antimycotic drugs and of a broad spectrum of antifungal plant constituents. Bio-autography can be considered as the most efficient assay for the detection of antimicrobial compounds because it allows the localization of the activity even in a complex matrix and thus a target-directed isolation of the active constituents possible (Rahalison *et al.*, 1993).

Test on *C. cucumerinum*

Preparation of the sample

The extracts were concentrated in a rotary evaporator and lyophilized before adding 1 ml of the solvent of extraction in 10 mg of each sample to give a concentration of 10 mg/ml. All samples are freshly prepared prior to the bioassay. For isolated compounds which showed activity at 20 µg or less were subsequently investigated against the same fungus in classical dilution experiments (Barry, 1980).

Thin layer chromatography

Silica-gel G60 F_{254} coated Al sheets (Merck) were used. The volume spotted on the TLC plate was 10 µl, corresponding to 100 µg of extract. For active extracts, the assays were repeated in duplicate at a lower sample amount (50 µg). The following mobile phases were used for the separation: Pet. ether-EtOAc (1:1) for Pet. spt., EtOAc and acetone extracts and $CHCl_3$:MeOH:H_2O (65:35:05) for MeOH extracts. TLC were run in duplicate of which one plate was used for the bioassay, whereas the second plate serving as reference chromatogram was stained with Godin reagent (Godin, 1954).

Bio-autography

Direct biography with *C. cucumerinum* was performed on Al-backed TLC sheets according to Homans and Fuchs, (1970) and Gottstein *et al.,* (1984). After developing the TLC plates they were dried well to remove the solvent and UV active spots were detected at 254 and 366 nm. A suspension of the spores of the fungus *C. cucumerinum* (prepared with CIBA samples) sprayed over the plates and kept at a room temperature for two days in polythene boxes with water. The grayish colour created by the grown fungus on the plates flushed the inhibition zones clear. To kill the fungus on TLC plates EtOH solution is sprayed. All these activities have been done according to the successful screening formulation applied by Rahalison *et al.,* (1993).

Direct bio-autography for antimicrobial test is very sensitive and gives accurate localization of active compounds and therefore permits a target-directed isolation of the active constituents (Rahalison *et al.,* 1991). For simple comparison with the inhibiting compounds present in the sample, sample and compounds can be used on the same plate as a reference (Rahalison, 1994).

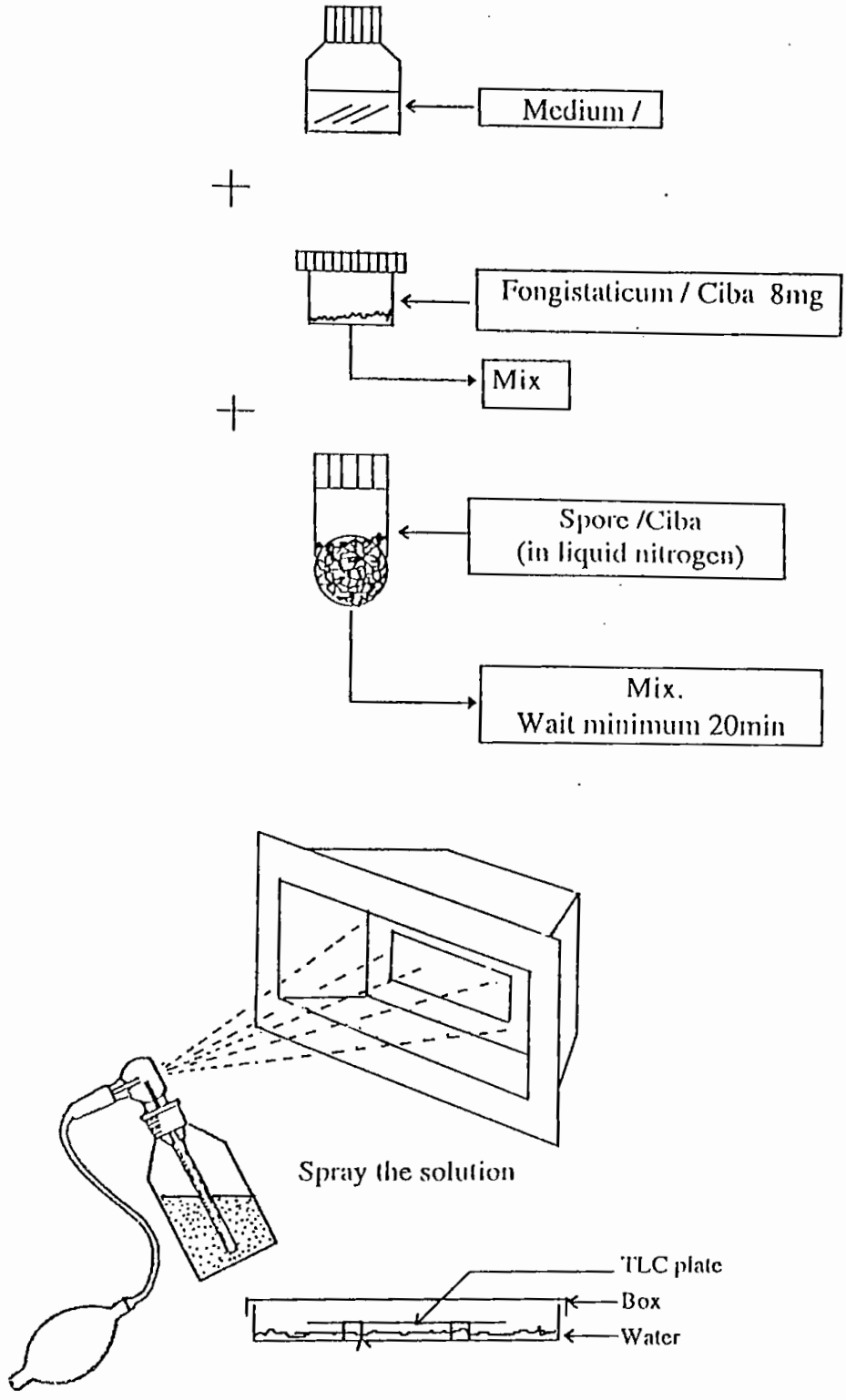


Fig. 9. Preparation and setting of fungicidal test.

Test on *C. albicans*

Preparation of the sample

Concentrated extracts were lyophilized and 1 ml of the solvent of extraction added in a 10 mg material to give a concentration of 10 mg/ml. Samples were prepared prior to the application.

Thin layer chromatography

Silica gel G60 F_{254} glass-backed plates were used. Dilution corresponding to 100 µg of extract and fractions were applied. The TLC were developed with the solvent systems: petroleum ether:EtOAc (1:1) for Pet. spt., EtOAc and acetone extracts and $CHCl_3$:MeOH:H_2O (65:35:5) for MeOH extracts. Chromatograms were dried with a hair dryer for complete removal of solvents. All TLC plates were run in duplicate. One of them being used as the reference chromatogram. UV active spots were detected at 254 and 366 nm. The reference chromatogram were stained with Godin reagent (Godin, 1954).

Culture media

Sabouraud liquid medium (Diagnostic Pasteur, Marnes La coquette, France) was used for the culture of *C. albicans* yeasts were maintained on sabouraud agar slants. All media were autoclaved at 120°C for 20 min. In order to obtain an exponential growth phase of *C. albicans* the sabouraud broth (20 ml) was inoculated 5-6 h before the test. The cultures were shaken at room temperature on a rotary shaker at 200 rpm.

Inoculation for the assay

Malt agar (Oxoid) for *C. albicans* was used as the solid media for the overlays. The molten media were maintained in a water bath at 45°C. The optical density of 600

nm (OD_{600}) of the *C. albicans* culture was measured with a UV/VIS spectrophotometer (an OD_{600} equal to 1 corresponds to approx. 10^7 cells/ml). The final concentration in the solid medium was approx. 10^5 cells/ml. The suspension was prepared immediately before carrying out the test.

Bio-autography

Chromatograms were placed on a hot plate maintained at 35°C. Approx. 10 ml of the inoculum was rapidly distributed over the TLC plate (10×5 cm) with a sterile pipette. After solidification of the medium, TLC plates were incubated overnight at 30°C in polythene boxes lined with moist chromatographic paper. The bioautograms were sprayed with an aqueous solution (2.5 mg/ml of thiazolyl blue (methylthiazolyl tetrazolium chloride, MTT, Fluka), and incubated for 4 h at 30°C. Clear inhibition zones were observed against a purple background.

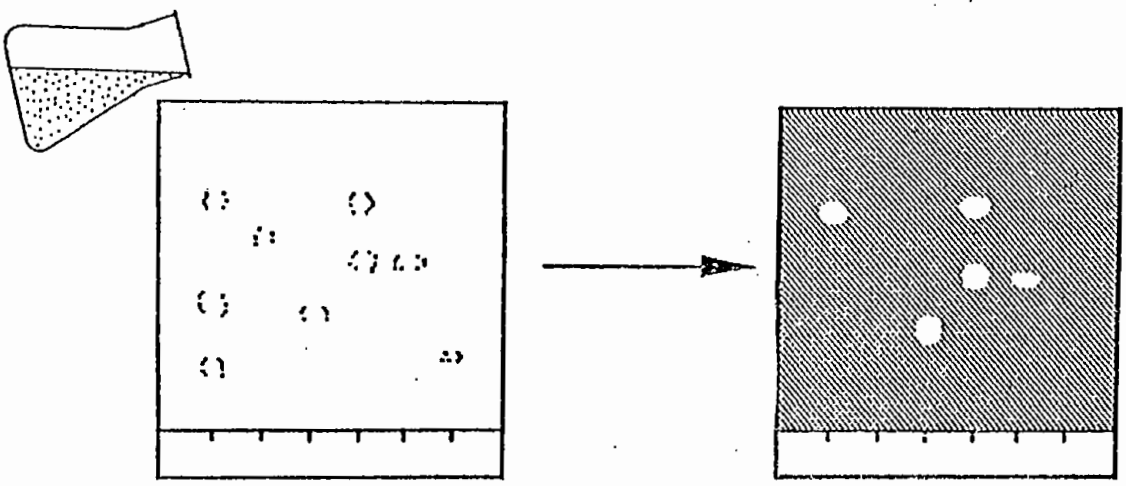


Fig. 10. Application of agar-overlay and the visible inhibition zones.

2.2.5. Bacteriocidal test

Activity against *B. subtilis* was assessed on glass-backed TLC plates in an agar overlay assay (Rahalison *et al.*, 1991). Propiconazole (Ciba) and miconazole (Janssen) were used as possible controls.

Preparation of the sample

The extracts were concentrated in a rotary evaporator and lyophilized before adding 1 ml of the solvent of extraction in 10 mg of each sample to give a concentration of 10 mg/ml. All samples were freshly prepared prior to the bioassay.

Culture media

B. subtilis strains were lyophilized or maintained on Luria-Bertani (LB) agar slants (bacto-tryptone 10 g/L, yeast extract 5 g/L, NaCl 10 g/L, bacto-agar 15 g/L). Cultures were grown in LB broth (same composition but without bacto-agar). All media were autoclaved at 120°C for 20 min. *B. subtilis* was grown in LB broth about 8 h before the test. The cultures were shaken at room temperature on a rotary shaker at 200 rpm.

Inoculum for the assay

LB agar for *B. subtilis* was used as the solid media for the overlays. The molten media were maintained in a water bath at 45°C. The suspension of *B. subtilis* was prepared at a final concentration of approx. 10^8 cells/ml (OD_{600} =1 for the bacteria). The suspension was prepared immediately before carrying out the test.

Thin layer chromatography

Silica gel G60 F_{254} glass-backed plates were used. Dilution corresponding to 100 µg of plant extract and fractions were applied. The TLC were developed with the following solvent systems: Petroleum ether:EtOAc (1:1) for Pet. spt, EtOAc and acetone extract and $CHCl_3$:MeOH:H_2O, (65:35:05) for MeOH extract. Chromatograms were dried with a hair dryer for complete removal of solvents. All TLC plates were run in duplicate. One of them being used as the reference chromatogram. UV active spots were detected at 254 and 366 nm. The reference chromatograms were stained with Godin reagent (Godin, 1954). For active extracts, the assays were repeated in duplicate at a lower sample amount (50 µg). The mixture of equal volume of the ethanolic solution of vanillin 1% and water solution of 3% parchloric acid. After spraying the TLC plate with this reagent ethanolic solution of 10% H_2SO_4 is sprayed and dried with 100°C with a hair dryer.

Bio-autography

Chromatograms were placed on a hot plate maintained at 35°C. Approx. 10 ml of the inoculum was rapidly distributed over the TLC plate (10×5 cm) with a sterile pipette. After solidification of the medium, TLC plates were incubated overnight at 30°C in polythene boxes lined with moist chromatographic paper. The bioautograms were sprayed with an aqueous solution (2.5 mg/ml) of thiazolyl blue (Methyl thiazalyl tetrazolium chloride; MTT (Fluka), and incubate for 4 h at 30°C. Clear inhibition zones were observed against a purple background.

2.3. Fractionation techniques

2.3.1 General pathways

The key to any successful program involving the investigation of biologically active plant constituents is the availability and choice of chromatographic techniques for the separation of pure substances. The aim is to have maximum yield with minimum effort (to reduce the time and cost of the separation procedure). Preparative separation techniques can be tedious and time consuming, especially when complex mixtures, such as, crude plant extracts have to be resolved. Over the past decade or so, several new techniques have been introduced, leading to the acceleration and simplification of different separation problems (Hostettmann *et al.*, 1986; Marston and Hostettmann, 1991; Hostettmann *et al.*, 1991). However, there is no universal technique capable of solving every isolation problem. All methods have advantages and limitations, so much, so that the best results are often obtained by a combination of two or more of these.

The most important preparative separation techniques employed in the isolation and purification of plant constituents are as follows:

Preparative separation methods for plant constituents

Solid phase chromatography
Paper chromatography
Preparative TLC, Centrifugal TLC
Open-column chromatography
Vacuum liquid chromatography
Pressure column chromatography, flash chromatography
Low-pressure liquid chromatography (LPLC)
Medium-pressure liquid chromatography (MPLC),
High-pressure liquid chromatography/High performance liquid chromatography(HPLC)
Liquid liquid chromatography
Craig distribution
Droplet countercurrent chromatography (DCCC)
Rotation locular countercurrent chromatography (RLCC)
Centurifugal partition chromatography (CPC)

Of the methods in the solid phase category, column chromatography is very popular and used extensively. It can include non-exchange resins, polymeric columns, gel-filtration, and chromatography over silica-gel or chemically modified silica-gel. Open column chromatography has a high load capacity but the separation time is long and the resolution respectively low.

To find the probable elution time and to check the purity of the isolated compounds HPLC, HP1090 Series II (Hewlett packard) has been used, while the column was Novapak C-18 (3.9×300 nm, 60A 4 µm). For the distillation of the HPLC organic solvents Fontavapor 210 (BÜCHI) 0.5 µm and for H_2O 0.45 µm (Millipore) were used. For the low pressure liquid chromatography the pump was Duramat-80 (Chemie un fitter, Regendrof), column Lichroprep® DIOL (Merk) 40-63 µm, detector LKG Unicord II and collector Bromma 2138 UVICORDS were used. HPTLC RP-18 WF_{254} S (Merck) was used for the selection of the solvent system for Lobar. Melting point (uncorrected) of the powder like one (compound 2) was determined with a mettler FP80 hot-stage apparatus. ^{1}H-and ^{13}C-NMR spectra were recorded with TMS as internal standard on a Varian VXR-200 spectrometer operating at 200 and 50 MHz respectively. Carbon multiplicities were established by DEPT experiments. Electron impact (EI) mass spectra were taken on a Nermag R 1030 / Tandem TSQ 700 (Finnigan MAT) mass spectrometer at 70 eV. The IR spectrum was taken by Perkin-Elmer 781 infrared spectrometer. The structures were readily determined from 1D (^{1}H and ^{13}C) and 2D (COSY, ^{1}H- detected direct, and long range ^{13}C-^{1}H correlations) NMR spectra recorded. The sticky one of the two isolated compounds showed activity at 20 µg as less was subsequently investigated against *C. cucumerinum* in Barry's (1980) classical dilution experiments.

2.3.2. Chromatographic methods

Different chromatographic methods have been utilized in this investigation to detect the presence of biologically active properties, to isolate the active ones given inhibition zones on the bioautograms and to purify the isolated components. Thin layer chromatography (TLC), High potential thin layer chromatography (HPTLC), Column chromatography, Low-pressure liquid chromatography (LPLC/Lobar), Medium-pressure liquid chromatography (MPLC) and High performance liquid chromatography (HPLC) were used in this regard.

2.3.2.1 Chromatography on TLC plates

For the normal phase chromatography Silica gel G60 F_{254} on Al sheets (Merck) were used. Ten mg/ml of the sample in the solvent of extract offered 100 µg/spot while spotted 10µl for each of the samples. The chromatograms then developed within a conventional chamber (Camag) with the following solvent systems:

Pet. spt. extract	Pet. ether:EtOAc (1:1)
EtOAc extract	do
acetone extract	do
MeOH extract	$CHCl_3$:MeOH:H_2O (65:35:5)

All chromatograms were observed under UV at 254 and 366 nm and marked with a pencil.

2.3.2.2. Godin reagent (Godin 1954)

The reagent is the mixture of the equal volume of 1% ethanolic solution of vanillin and 3% aqueous solution of perchloric acid. After spraying the reagent on the

dried TLC plate 10% ethanolic solution of H_2SO_4 is also spayed before drying the plate at 100°C to reveal the spots of the compounds.

2.3.2.3. Column Chromatography

For the open column chromatography, Si60 (63-200 mesh) and silica gel Si60 (230-400 mesh) (Merck) and glass column of different size (45×5 cm, 45×1.75 cm, 44×2.75 cm etc) were used. The cotton pad used at the base of the column were soaked in acetone and dried before application. Sea sand (Merck) was used at the top over the prepared sample to protect destruction of the sample layer. Dry application was made mixing the sample with a little amount of the given silica-gel and dissolved within required amount of the solvent of extraction (only to make the sample dissolved) and then dried in a rotary evaporator to give a powder. Different solvent systems were used as eluent. The elution rate was 1 ml/min. A fraction collector was used to collect the fractions.

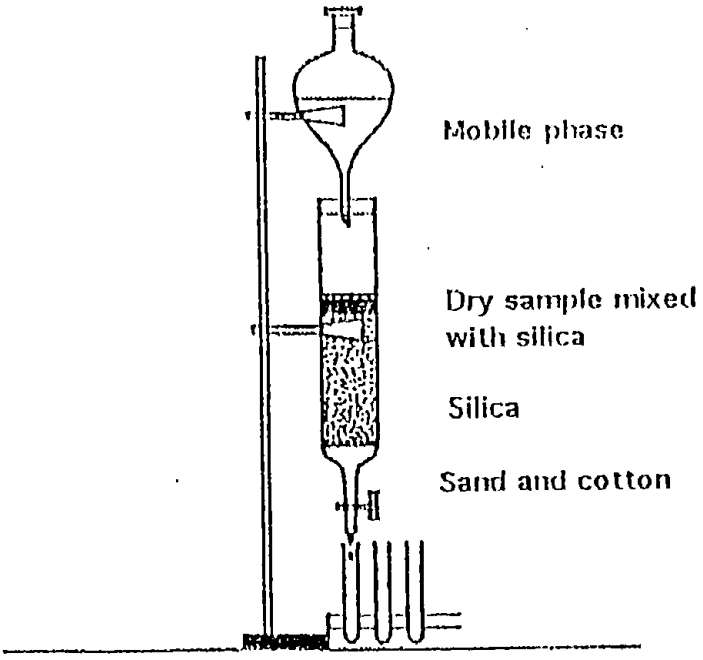


Fig. 11. Open column chromatography.

2.3.2.4. Gel filtration

Open columns are used using sephadex LH-20 (Pharmacia) for the chromatography of exclusion. The separation of the methanolic extracts done with MeOH using as the eluent and other lipophilic extracts also with MeOH with a little amount (not more than 50%) of $CHCl_3$. The eluent allowed about 0.5 ml/min.

2.3.2.5. Low-pressure liquid chromatography

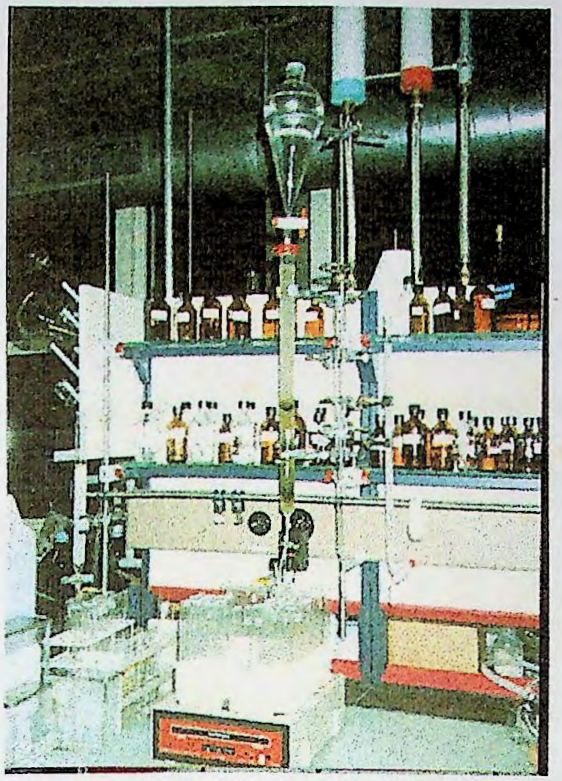
Low-pressure liquid chromatography was used with lobar columns (Merck) with a pump Duramat-80 (Chemie and Filter Regensdrof). The pressure was environ 1-3 bar. The columns were (31×2.5 d.i.) containing silica-gel (40-63 µm) or reverse phase (40-63 µm) RP-8, PR-18 or Diol with the help of a detector LKB 2010.

2.3.2.6. Medium-pressure liquid chromatography

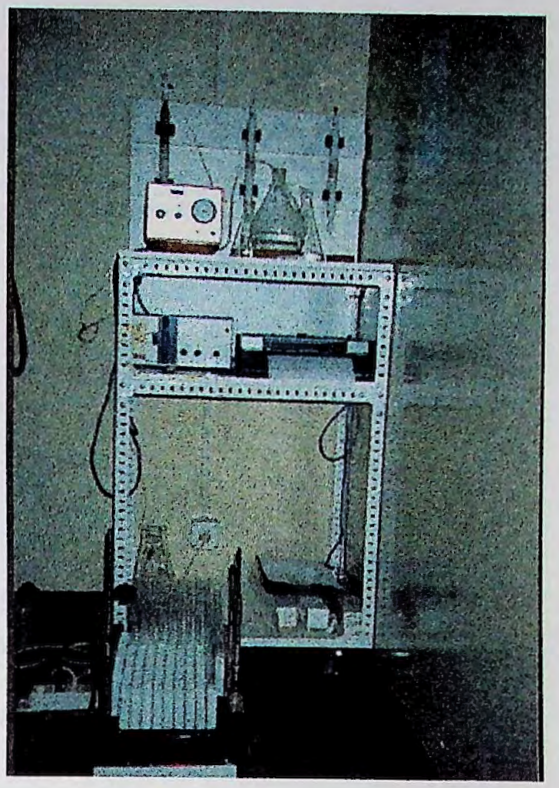
The medium-pressure liquid chromatography was used with a pressure below 20 bar and silica gel Si60 230-400 mesh (ASTM) was used. A detector was also used to record the spectra.

2.3.2.7. High performance liquid chromatography

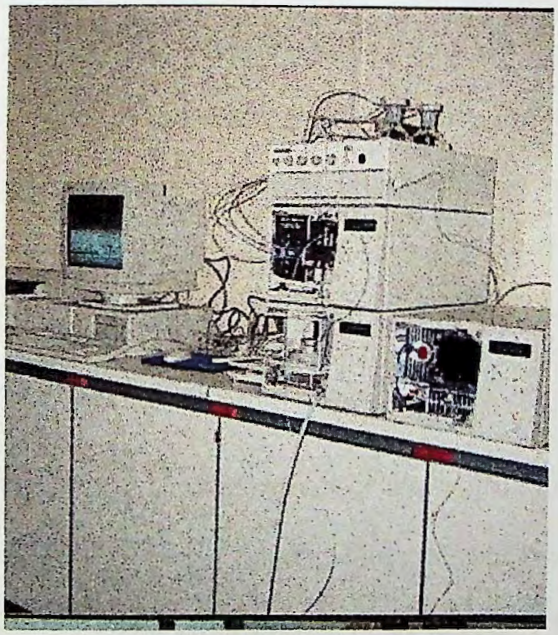
For the analytic HPLC the equipment used were, pump- Spectra physics 8700; Injector- Rheodyne; Detector- HP 1090 (Hewlett Packard); Columns-Novapak C-18.



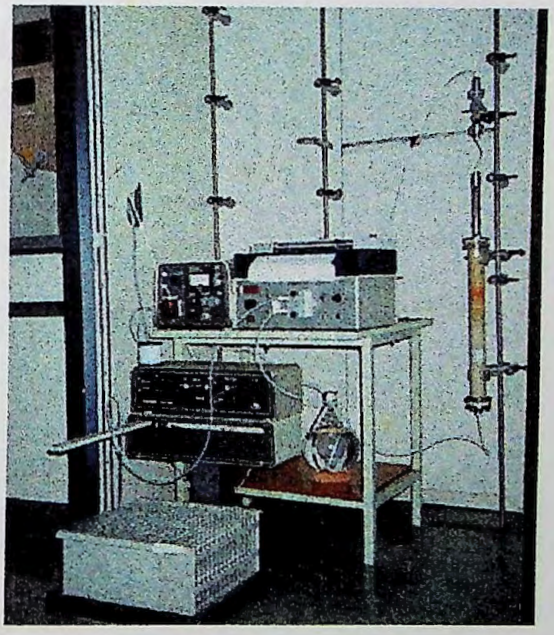
Open column chromatography



LPLC



HPLC



MPLC

Fig. 12. Chromatographic systems applied in this investigation.

2.4. Isolation of bio-active principles

Air-dried leaves, seeds, fruit-pericarp, stem-bark and roots of *A. rohituka* were pulverised with liquid nitrogen in a Retsch mill and extracted at room temperature successively with Pet. spt., EtOAc, acetone and MeOH giving a 72 hours reflux for each of the items separately. Extracts were filtered and concentrated in a rotary evaporator under reduced pressure, and after lyophilization twenty samples were collected in vials. In comparison to the dry weight of the plant materials percent output was recorded weighing the collected extracts.

Silica-gel GF_{254} coated Al sheets (Merck) and glass-backed plates (Merck) were used for antimicrobial tests. All samples were freshly prepared prior to the bioassay. The volume spotted on the TLC was 10 µl, corresponding to 100 µg of extract. In repetition of the bioassays the volume spotted on TLC was 200 µg to check and trace out the weak activity. Leaf extracts shown more activity, and especially the EtOAc extract of leaves were active on all the test organisms. For activity guided fractionation EtOAc extracts of *A. rohituka* leaves was selected regarding the activity against *C. cucumerinum*. Test samples were prepared in favour of each of the extract type with a concentration of 10 mg /ml where Pet. spt., EtOAc and acetone extracts were dissolved in $CHCl_3$ and MeOH extract in MeOH.

The EtOAc extract of leaves (3.5 gm) was taken for bioassay guided fractionation on a 45.5× 5 cm open column of silica gel Si60 63-200 mesh (ASTM) with a mobile phase of Pet. ether:EtOAc (1:1) to afford 64 fractions. All fractions were then subjected to bioassay regarding the activity against *C. cucumerinum*, *B. subtilis* and *C. albicans* and two major batches were prepared, Fr. (14-20) as batch 'A' and Fr. (28-43) batch 'B'. After

removing the solvent *in vacuo*, the first 'A' batch yielded 230 mg and the second batch 'B' yielded 700 mg after lyophilization.

The solubility of the yields of the two batches 'A'. and 'B' were tested and it was found that both of them were soluble in MeOH, EtOAc separately and in a mixture of Pet. ether and EtOAc.

2.5. Purification of the isolated compounds

First batch, 'A'

Batch-'A' yield (230 mg) was subjected to purify on a LH-20 open column (45×1.75 cm) with 100% MeOH as eluent, which gave no good separation and offered a loss of 35 mg of the product. The rest 195 mg was then set on a silica-gel Si60 230-400 mesh (ASTM) open column (44×2.75 cm) with a mobile phase $CHCl_3$:MeOH:H_2O, (95:05:01) to give (Fr. 35-46) 106 mg of the expected product (confirmed over bioassay) with little impurity. The sample was then repeated with the same size (44×2.75 cm) silica-gel Si60 230-400 mesh (ASTM) open column with a changed mobile phase of $CHCl_3$:MeOH:H_2O, (98:02:01) to afford three sub-batches Fr. Fr. 92-101 (15 mg), Fr. 102+103 (9.9 mg), and Fr. 104-128 (5.5 mg). With a trace impurity 9.9 mg output was then subjected to purify over LH-20 (42×2 cm) open column with 100% MeOH as a mobile phase to offer (Fr. 35-40) 8.5 mg of the product after evaporation on rotavapor and complete lyophilization. This is **compound 1**, and it was a colourless sticky substance highly active on *C. cucumerinum*.

Second batch, 'B'

Batch-'B' yield (700 mg) was subjected to fractionation to find a compound active on *B. subtilis* and *C. albicans*. Using MPLC for silica-gel Si60 230-400 mesh (ASTM) with a mobile phase Pet. ether:EtOAc (1:1) no perfect fraction was adopted, while the whole mixture was evaporated to get back the extract and was applied on diol column on lobar with a mobile phase of MeOH 100% which offered a less satisfactory result. The material then subjected to purify on LH-20 (45×1.75 cm) open column with $CHCL_3$:MeOH (7:3) to afford 280 (Fr. 57-99) gm of the product with little impurity. On an open column of silica-gel Si60 230-400 mesh (ASTM) with $CHCl_3$-MeOH-H_2O (70:35:01) as a mobile phase it was purified having 78 mg (Fr. 36-42) as **compound 2.**

One more fraction was also purified further by gel filtration and crystallized in hexane:iso-propanol; (1:1) mixture to yield the same compound which was compared with a simple TLC spotting the both to check same Rf values and colouration (violet) for both of them after revelation with Godin reagent.

The schematic pathway of isolation given below where the used apparatus and the amount yield is mentioned:

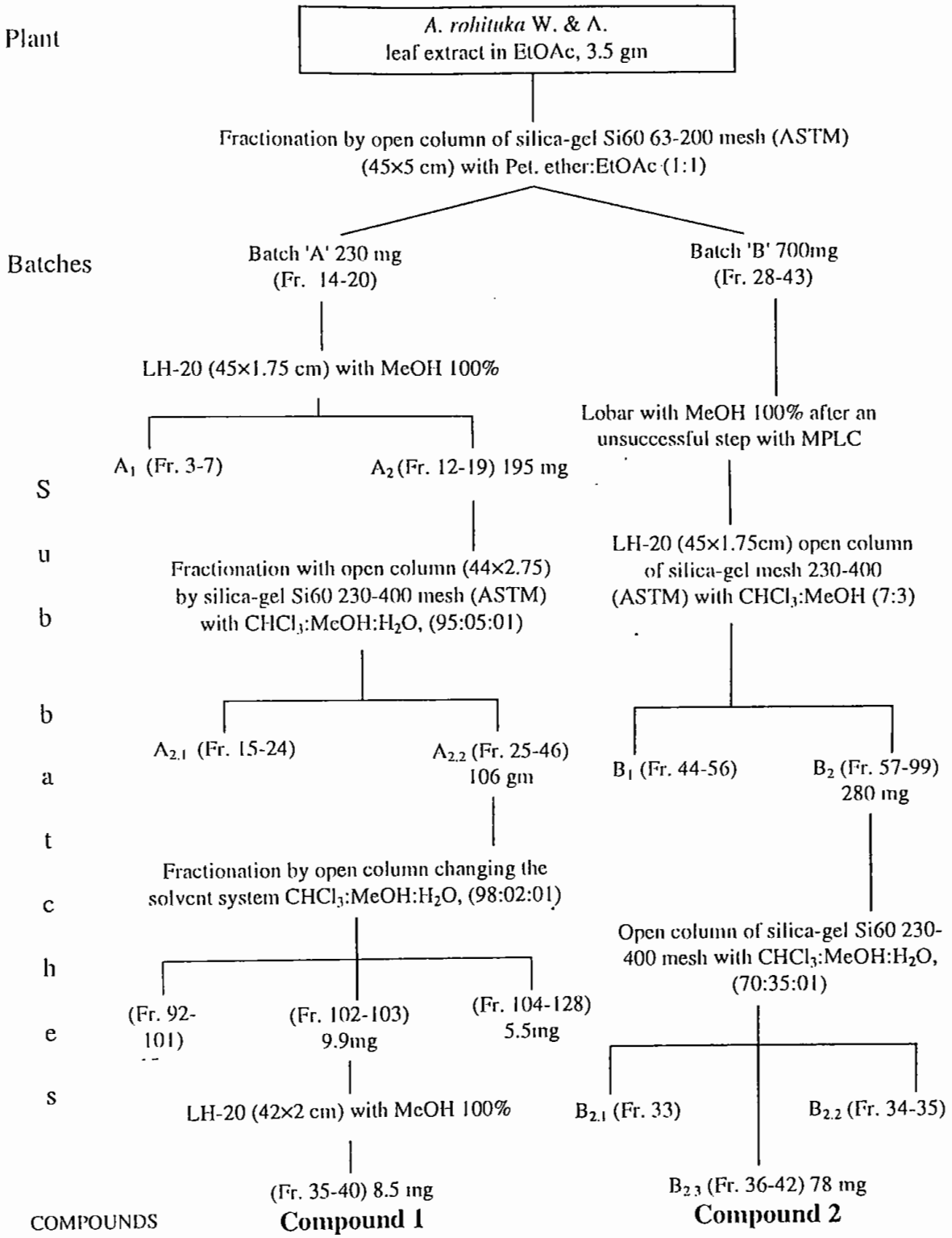
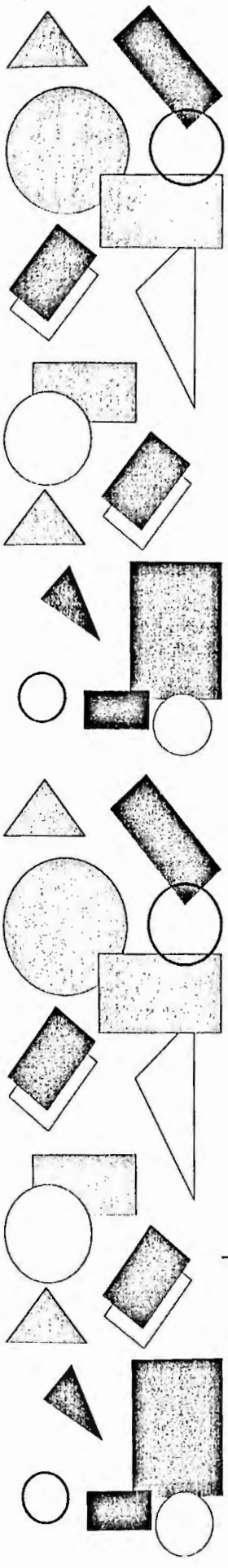


Fig. 13. Pathway of isolation of the two found compounds.



Chapter 3

Results

Chapter 3 Results

3.1. Output extracts

The extracts evaporated and lyophilized were sufficient to go through fractionation. A extract checklist including per cent collection is given in table 5:

Plant parts	Solvents used	Wt. of dust	Wt. of extract	% collection
Leaves	Pet. spt.	113.50	8.81	7.76%
	EtOAc	104.69	5.01	4.79%
	acetone	99.68	3.61	3.62%
	MeOH	96.07	9.66	10.06%
Seeds	Pet. spt.	100.84	26.18	25.96%
	EtOAc	74.66	12.59	16.86%
	acetone	62.07	3.64	5.86%
	MeOH	58.43	2.34	4.00%
Fruit-pericarp	Pet. spt.	100.00	5.28	5.28%
	EtOAc	94.72	11.90	1.26%
	acetone	82.82	1.95	2.35%
	MeOH	80.87	3.47	4.29%
Stem-bark	Pet. spt.	152.39	1.43	0.94%
	EtOAc	150.96	2.51	1.66%
	acetone	148.45	10.43	7.03%
	MeOH	138.02	12.28	8.90%
Root	Pet. spt.	111.00	0.88	0.79%
	EtOAc	110.12	1.72	1.56%
	acetone	108.40	0.79	0.73%
	MeOH	107.61	16.29	15.14%

Table 5. A checklist of output extracts.

3.2. Activity against red flour beetle *T. castaneum* strains

3.2.1. Activities of the extracts from leaves

Results of topical application of the leaf extracts in different concentration applied on three strains of the red flour beetle *T. castaneum* in the dose-mortality experiments are given in tables 6-9. Highest mortality found for EtOAc extracts of leaves, where LD_{50} values were ranging between 0.387 µg/µl (on FSS-II adult for 48 h) to 137.380 µg/µl (on CTC-12 adult for 48 h) and 14.392 µg/µl (on CR-1 pupae for 72 h) to 233.484 µg/µl (on CR-1 pupae for 24 h) (Table-7).

No significant χ^2 value and no recovery was found in case of leaf, in any of the collected extracts.

Mortality of the red flour beetle *T. castaneum* recorded in case of EtOAc and Pet. spt. extract which tends to highlight that there may have bio-active compounds present in the leaves. The diterpene alcohol **aphanamixol** was found neutral (Chandrasekharan and Chakrabortty, 1968). The alkaliod **rohitukine** was reported from the EtOH extract of the leaves and stems which was an alkaloid (Harmon *et al.*, 1979), but no information was available on its activity. From the closely related species *A. grandifolia* amoorastatone (isomeric with that of amoorastatin) and 12-hydroxy amoorastatin (two limonoids) were reported from the leaves mentioning there activities on Murine P-388 lymphocytic leukemia cell line that caused growth inhibition (Pettit *et al.*, 1983; Polonsky *et al.*, 1979).

Table 6. Activity of Pet. spt. extract of *A. rohituka* leaves against *T. castaneum.*

Strain	Life stage	Exposure time (h)	LD_{50} value µg/µl	Regression equation	χ^2 value
CR-I	Pupae	24	55.666	y = 1.526 + 1.990x	0.326
		48	48.436	y = 1.921 + 1.827x	0.193
		72	46.675	y = 1.732 + 1.958x	0.637
	Adult	24	24.805	y = 2.403 + 1.862x	0.856
		48	23.832	y = 2.426 + 1.869x	0.669
		72	21.814	y = 2.679 + 1.734x	3.067
FSS-II	Pupae	24	89.671	y = 2.922 + 1.064x	0.220
		48	53.104	y = 3.090 + 1.107x	0.337
		72	47.818	y = 3.235 + 1.051x	1.500
	Adult	24	15.958	y = 3.540 + 1.214x	1.214
		48	14.491	y = 3.426 + 1.356x	1.243
		72	11.489	y = 3.457 + 1.455x	1.606
CTC-12	Pupae	24	859.175	y = 2.909 + 0.713x	0.045
		48	423.110	y = 2.787 + 0.847x	0.106
		72	330.850	y = 3.094 + 0757x	0.182
	Adult	24	224.938	y = 3.107 + 0.805x	0.216
		48	39.063	y = 3.732 + 0.797x	0.059
		72	27.529	y = 3.807 + 0.829x	0.453

Table 7. Activity of EtOAc extract of *A. rohituka* leaves against *T. castaneum.*

Strain	Life stage	Exposure time (h)	LD_{50} value µg/µl	Regression equation	χ^2 value
CR-I	Pupae	24	233.484	y = 2.966 + 0.859x	0.243
		48	100.859	y = 3.789 + 0.605x	0.369
		72	14.392	y = 4.359 + 0.554x	0.109
	Adult	24	1.947	y = 4.682 + 1.099x	0.057
		48	1.435	y = 4.827 + 1.100x	0.276
		72	0.977	y = 5.010 + 0.960x	0. 267
FSS-II	Pupae	24	76.187	y = 3.115 + 1.002x	0.599
		48	77.095	y = 4.088 + 0.483x	0.310
		72	17.060	y = 3.455 + 1.254x	1.639
	Adult	24	0.958	y = 5.009 + 0.472x	0.033
		48	0.387	y = 5.170 + 0.412x	0.141
		72	2.347	y = 4.533 + 1.261x	0.125
CTC-12	Pupae	24	130.059	y = 1.486 + 1.662x	1.369
		48	141.275	y = 2.675 + 1.081x	0.044
		72	68.969	y = 4.023 + 0.531x	0.282
	Adult	24	130.059	y = 1.486 + 1.662x	1.369
		48	137.380	y = 2.251 + 1.286x	0.015
		72	81.176	y = 3.917 + 0.567x	0.236

Table 8. Activity of acetone extract of *A. rohituka* leaves against *T. castaneum.*

Strain	Life stage	Exposure time (h)	LD_{50} value µg/µl	Regression equation	χ^2 value
CR-I	Pupae	24	141.103	y = 2.714 + 1.064x	1.400
		48	77.647	y = 2.690 + 1.222x	0.841
		72	66.697	y = 3.040 + 1.075x	0.993
	Adult	24	64.302	y = 2.152 + 1.575x	0.119
		48	33.563	y = 2.305 + 1.766x	1.382
		72	47.744	y = 2.874 + 1.266x	2.153
FSS-II	Pupae	24	33.946	y = -2.578 + 4.950x	0.021
		48	36.205	y = 1.131 + 2.482x	0.948
		72	47.634	y = 2.580 + 1.443x	0.361
	Adult	24	312.583	y = 2.814 + 0.876x	0.486
		48	142.919	y = 2.709 + 1.063x	0.534
		72	322.477	y = 3.095 + 0.759x	0.008
CTC-12	Pupae	24	621.396	y = 2.349 + 0.949x	0.068
		48	17799.5	y = 3.240 + 0.414x	0.089
		72	506.036	y = 2.685 + 0.856x	0.339
	Adult	24	1472.383	y = 2.988 + 0.635x	0.826
		48	170.607	y = 2.758 + 1.004x	0.659
		72	75.546	y = 2.241 + 1.469x	0.032

Table 9. Activity of MeOH extract of *A. rohituka* leaves against *T. castaneum*.

Strain	Life stage	Exposure time (h)	LD_{50} value µg/µl	Regression equation	χ^2 value
CR-1	Pupae	24	609.340	y = 1.774 + 1.158x	0.352
		48	5344.116	y = 3.315 + 0.452x	0.007
		72	181.986	y = 3.258 + 0.771x	1.362
	Adult	24	336.122	y = 2.805 + 0.869x	0.145
		48	169.043	y = 3.808 + 0.535x	0.433
		72	169.789	y = 3.833 + 0.523x	0.923
FSS-II	Pupae	24	718.672	y = 1.231 + 1.319x	0.454
		48	457.895	y = 2.010 + 1.124x	0.143
		72	70.363	y = 2.604 + 1.297x	1.065
	Adult	24	85.068	y = 2.561 + 1.264x	2.683
		48	106.651	y = 3.239 + 0.868x	0.134
		72	94.067	y = 3.302 + 0.861x	0.597
CTC-12	Pupae	24	4048.622	y = 2.230 + 0.768x	0.166
		48	5457.713	y = 3.117 + 0.504x	0.232
		72	576.568	y = 3.295 + 0.617x	0.145
	Adult	24	6207.093	y = 3.658 + 0.354x	0.178
		48	514.327	y = 3.275+ 0.636x	0.200
		72	494.789	y = 3.175 + 0.677	0.056

3.2.2. Activity of the extracts from seeds

The mortality of the insects in case of EtOAc extract of seeds given in tables 10-13. All the extracts were with a orange-red color. Highest pupal mortality was recorded in case of MeOH extract of seeds (Table-13) for all the strains.

Significant χ^2 values (4.657 and 4.818) at 1% level of probability for 2 degrees of freedom was found for the EtOAc and acetone extract on CTC-12 pupae for 72 h and CTC-12 adults after 48 h of treatment (Table-11, 12), while the LD_{50} values were 87.009 µg/µl and 14.530 µg/µl and the regression equations were y = 0.802 + 2.164x and y= 3.015 + 1.078x respectively.

No-recovery was found in case of seed extracts applied on both pupae and adults of three different strains of *T. castaneum.*

From the seeds all reported limonoids were bio-active. **Rohitukin** was isolated from EtOH extract (Connolly *et al.*, 1976; Lidert *et al.*, 1985), and there was a report on its activity that it inhibits Murine P-388 lymphocytic leukemia at LD_{50}=100; T/C inactive (2→0.25 mg) (Pettit, *et al.*, 1983) tested at the National Cancer Institute. **Rohituka-7** (prieurianin-type limonoid) was also reported from the seeds which is antifeedant against tobacco budworm (Lidert *et al.*, 1985). The saponin, **stigmasta-5, 24 (28)-dien 3β-*O*-β-D glucopyranosyl-*O*-α-L-rhamnopyranoside** was also isolated from the seeds of *A. rohituka* (Bhatt *et al.*, 1981), but nothing was informed about its activity. Two other limonoids aphanastatin and amoorastatin were also found strong to inhibit growth of Murine P-388 lymphocytic leukemia (Polonsky *et al.*, 1978a,b; Pettit *et al.*, 1983), which were reported from the seeds of the related species *A. grandifolia.*

Table 10. Activity of Pet. spt. extract of *A. rohituka* seed against *T. castaneum.*

Strain	Life stage	Exposure time (h)	LD_{50} value µg/µl	Regression equation	χ^2 value
CR-I	Pupae	24	488.215	y = 3.048 + 0.726x	0.289
		48	213.384	y = 3.223 + 0.763x	0.455
		72	37.120	y = 3.863 + 0.725x	0.141
	Adult	24	30.283	y = 4.200 + 0.540x	0.291
		48	38.530	y = 4.052 + 0.598x	0.449
		72	36.678	y = 3.854 + 0.732x	0.410
FSS-II	Pupae	24	203.049	y = 3.547+ 0.630x	0.167
		48	103.773	y = 3.169 + 0.908x	0.054
		72	37.090	y = 3.011 + 1.268x	0.524
	Adult	24	17.458	y = 4.296 + 0.567x	0.556
		48	22.624	y = 3.755 + 0.919x	1.619
		72	21.309	y = 3.794 + 0.908x	1.228
CTC-12	Pupae	24	230.742	y = 2.555 + 1.035x	0.023
		48	150.081	y = 2.949 + 0.942x	0.205
		72	72.079	y = 2.866 + 1.149x	1.599
	Adult	24	61.694	y = 3.519 + 0.827x	0.206
		48	36.595	y = 3.951 + 0.671x	0.043
		72	15.614	y = 4.416 + 0.489x	0.095

Table 11. Activity of EtOAc extract of *A. rohituka* seed against *T. castaneum*.

Strain	Life stage	Exposure time (h)	LD_{50} value µg/µl	Regression equation	χ^2 value
CR-1	Pupae	24	163.537	y = 2.755 + 1.014x	0.172
		48	109.922	y = 1.478 + 1.725x	0.887
		72	72.763	y = 2.690 + 1.241x	1.046
	Adult	24	2.756	y = 4.641 + 0.816x	0.551
		48	1.309	y = 4.915 + 0.729x	0.362
		72	7.454	y = 3.654 + 1.543x	0.026
FSS-II	Pupae	24	280.920	y = 1.238 + 1.536x	0.243
		48	108.456	y = 0.245 + 2.336x	0.660
		72	92.612	y = 0.776 + 2.148x	0.592
	Adult	24	0.789	y = 5.058 + 0.566x	0.027
		48	5.206	y = 4.203 + 1.113x	0.046
		72	3.311	y = 4.526 + 0.912x	0.121
CTC-12	Pupae	24	139.762	y = 0.703 + 2.003x	1.914
		48	112.314	y = 1.930 + 1.497x	1.913
		72	87.009	y = 0.802 + 2.164x	4.657
	Adult	24	1.073	y = 4.982 + 0.570x	0.742
		48	1.779	y = 4.816 + 0.737x	0.955
		72	1.308	y = 4.908 + 0.793x	0.035

Table 12. Activity of acetone extract of *A. rohituka* seed against *T. castaneum*.

Strain	Life stage	Exposure time (h)	LD_{50} value µg/µl	Regression equation	χ^2 value
CR-1	Pupae	24	93.361	y = 3.938 + 0.539x	0.025
		48	20.531	y = 3.176 + 1.390x	1.234
		72	10.430	y = 3.560 + 1.414x	0.857
	Adult	24	20.091	y = 2.387 + 2.005x	1.681
		48	11.883	y = 3.206 + 1.669x	0.168
		72	11.495	y = 3.135 + 1.759x	0.380
FSS-II	Pupae	24	28.150	y = 3.152 + 1.275x	3.881
		48	17.664	y = 2.140 + 2.293x	0.059
		72	13.839	y = 2.429 + 2.253x	2.322
	Adult	24	21.445	y = 2.500 + 1.878x	0.109
		48	14.962	y = 2.365 + 2.242x	1.049
		72	14.790	y = 2.278 + 2.326x	0.884
CTC-12	Pupae	24	36.033	y = 3.804 + 0.768x	0.255
		48	11.807	y = 4.009 + 0.924x	1.474
		72	9.785	y = 4.096 + 0.913x	0.524
	Adult	24	17.790	y = 3.190 + 1.448x	1.785
		48	14.530	y = 3.015 + 1.708x	4.818
		72	14.418	y = 3.453 + 1.335x	2.411

Table 13. Activity of MeOH extract of *A. rohituka* seed against *T. castaneum.*

Strain	Life stage	Exposure time (h)	LD_{50} value µg/µl	Regression equation	χ^2 value
CR-I	Pupae	24	131.361	y = 3.813 + 0.561x	0.175
		48	14.894	y = 3.918 + 0.922x	0.042
		72	12.738	y = 3.822 + 1.066x	0.122
	Adult	24	109.043	y = 3.052 + 0.956x	0.244
		48	140.326	y = 3.146 + 0.864x	0.358
		72	95.102	y = 3.412 + 0.803x	0.600
FSS-II	Pupae	24	118.675	y = 3.948 + 0.507x	0.175
		48	13.185	y = 4.130 + 0.777x	0.018
		72	8.792	y = 4.162 + 0.887x	0.128
	Adult	24	218.809	y = 3.582 + 0.606x	0.349
		48	173.752	y = 3.686 + 0.587x	0.009
		72	61.318	y = 3.655 + 0.752x	0.128
CTC-12	Pupae	24	61.182	y = 3.953 + 0.586x	0.155
		48	18.285	y = 3.795 + 0.954x	0.098
		72	13.483	y = 4.010 + 0.876x	0.055
	Adult	24	121.442	y = 2.863 + 1.025x	0.868
		48	107.253	y = 3.207 + 0.883x	0.634
		72	75.458	y = 3.623 + 0.734x	1.107

3.2.3. Activity of the extracts from the fruit-pericarp

Results of the dose-mortality experiments on the pupae and adults of the *T. castaneum* strains with the fruit-pericarp extracts collected in Pet. spt., EtOAc, acetone and MeOH given in tables 14-17. Highest mortality was recorded for EtOAc extract of fruit-pericarp, where LD_{50} values were ranging between 0.451 µg/µl (on FSS-II pupae for 24 h) to 9.597 µg/µl (on CTC-12 pupae for 72 h) and 3.565 µg/µl (on FSS-II adults for 24 h) to 15.985 µg/µl (on CR-1 adult for 24 h) (Table-15), then Pet. spt. extract of fruit-pericarp, where LD_{50} values were ranging between 0.220 µg/µl (on CTC-12 pupae for 24 h) to 1.335 µg/µl (on FSS-II pupae for 48 h) and 1.011 µg/µl (on CTC-12 adult for 48 h) to 21.265 µg/µl (on CTC-12 adult for 24 h) (Table-14). Lowest mortality was also in case of this organ, i.e., MeOH extract of fruit-pericarp, where LD_{50} values were ranging between 14220.850 µg/µl (on FSS-II pupae for 24 h) to 10.614 µg/µl (on CR-1 pupae for 72 h) (Table-17).

Recoveries were recorded in case of Pet. spt. (CR-1 and CTC-12 adults for 48- to 72 h) extract of the fruit-pericarp, and it was always in case of adults. Pupal mortality was comparatively higher in cases of Pet. spt., EtOAc and MeOH extracts, while acetone showed parallel effect for both pupae and adults.

The major toxic constituent of *Amoora* present in the fruit-pericarp isolated as a terpenoid and named **aphanamixin** (Chatterjee and Kundu, 1967; Chatterjee *et al.*, 1970) which was found biologically active, LC_{50}= 0.06 ppm (48h), (Nishizawa *et al.*, 1984). It was also reported along with two other minor toxic constituents aphanamol I and aphanamol II from the fruit-peel of the related species *A. grandifolia* (Nishizawa *et al.*, 1984).

Table 14. Activity of Pet. spt. extract of *A. rohituka* fruit-pericarp against *T. castaneum*.

Strain	Life stage	Exposure time (h)	LD_{50} value µg/µl	Regression equation	χ^2 value
CR-1	Pupae	24	0.635	y = 5.145 + 0.737x	0.550
		48	0.774	y = 5.105 + 0.940x	0.541
		72	1.074	y = 4.960 + 1.281x	0.709
	Adult	24	1.795	y = 4.848 + 0.600x	0.320
		48	1.736	y = 4.731 + 1.119x	0.048
		72	2.256	y = 4.648 + 0.995x	0.314
FSS-II	Pupae	24	0.780	y = 5.100 + 0.925x	0.274
		48	1.335	y = 4.821 + 1.425x	0.386
		72	-	-	-
	Adult	24	8.583	y = 4.262 + 0.790x	0.084
		48	1.853	y = 4.827 + 0.647x	0.004
		72	3.566	y = 4.538 + 0.836x	0.049
CTC-12	Pupae	24	0.220	y = 5.337 + 0.512x	0.718
		48	0.466	y = 5.257 + 0.775x	0.097
		72	1.061	y = 4.965 + 1.358x	0.064
	Adult	24	21.265	y = 4.387 + 0.461x	0.755
		48	1.011	y = 4.998 + 0.355x	0.125
		72	3.840	y = 4.777 + 0.382x	0.073

Table 15. Activity of EtOAc extract of *A. rohituka* fruit-pericarp against *T. castaneum.*

Strain	Life stage	Exposure time (h)	LD_{50} value µg/µl	Regression equation	χ^2 value
CR-1	Pupae	24	1.743	y = 4.847 + 0.636x	0.399
		48	4.189	y = 4.224 + 1.248x	1.010
		72	1.527	y = 4.801 + 1.080x	0.047
	Adult	24	15.985	y = 3.789 + 1.006x	0.101
		48	12.003	y = 3.652 + 1.249x	0.463
		72	6.787	y = 4.163 + 1.007x	0.341
FSS-II	Pupae	24	0.450	y = 5.109 + 0.314x	0.092
		48	1.841	y = 4.857 + 0.539x	0.108
		72	5.502	y = 4.186 + 1.100x	0.895
	Adult	24	3.565	y = 4.725 + 0.498x	0.295
		48	5.160	y = 4.499 + 0.703x	0.018
		72	4.322	y = 4.491 + 0.801x	0.101
CTC-12	Pupae	24	9.253	y = 4.440 + 0.580x	0.376
		48	7.201	y = 4.334 + 0.777x	0.074
		72	9.597	y = 3.731 + 1.292x	0.023
	Adult	24	13.183	y = 4.034 + 0.862x	0.461
		48	6.580	y = 4.384 + 0.753x	0.313
		72	5.614	y = 4.389 + 0.815x	0.067

Table 16. Activity of acetone extract of *A. rohituka* fruit-pericarp against *T. castaneum*.

Strain	Life stage	Exposure time (h)	LD_{50} value µg/µl	Regression equation	χ^2 value
CR-1	Pupae	24	91.711	y = 2.913 + 1.063x	0.534
		48	129.795	y = 3.166 + 0.868x	0.890
		72	34.410	y = 3.383 + 1.052x	0.032
	Adult	24	99.945	y = 2.742 + 1.129x	0.031
		48	103.224	y = 3.175 + 0.906x	0.005
		72	99.990	y = 3.660 + 0.670x	0.011
FSS-II	Pupae	24	963.505	y = 3.321 + 0.563x	0.005
		48	264.255	y = 2.970 + 0.838x	0.560
		72	74.661	y = 3.575 + 0.761x	0.272
	Adult	24	65.555	y = 2.698 + 1.267x	0.260
		48	253.000	y = 3.520 + 0.616x	0.028
		72	59.848	y = 3.372 + 0.916x	0.870
CTC-12	Pupae	24	830.940	y = 3.324 + 0.574x	0.026
		48	641.801	y = 3.334 + 0.594x	0.034
		72	49.283	y = 3.500 + 0.888x	0.199
	Adult	24	232.045	y = 3.370 + 0.689x	0.262
		48	61.121	y = 3.803 + 0.670x	0.212
		72	53.211	y = 3.511 + 0.863x	0.912

Table 17. Activity of MeOH extract of *A. rohituka* fruit-pericarp against *T. castaneum*.

Strain	Life stage	Exposure time (h)	LD_{50} value µg/µl	Regression equation	χ^2 value
CR-1	Pupae	24	2088.810	y = 3.481 + 0.458x	0.137
		48	772.637	y = 3.779 + 0.423x	0.391
		72	10.614	y = 4.152 + 0.827x	0.639
	Adult	24	6392.65	y = 3.232 + 0.465x	0.041
		48	620.004	y = 3.310 + 0.605x	0.262
		72	410.271	y = 3.509 + 0.571x	0.013
FSS-II	Pupae	24	14220.850	y = 3.686 + 0.316x	0.003
		48	276.343	y = 3.672 + 0.544x	0.061
		72	57.968	y = 3.940 + 0.601x	0.664
	Adult	24	2088.810	y = 3.481 + 0.458x	0.137
		48	1716.797	y = 3.439 + 0.483x	0.024
		72	2975.497	y = 3.673 + 0.382x	0.041
CTC-12	Pupae	24	2134.344	y = 3.706 + 0.389x	0.028
		48	42.950	y = 3.605 + 0.854x	0.268
		72	14.886	y = 3.883 + 0.952x	0.206
	Adult	24	324.784	y = 3.591 + 0.560x	0.408
		48	161.060	y = 3.412 + 0.720x	1.387
		72	98.596	y = 3.578 + 0.713x	1.214

3.2.4. Activity of the extracts from the stem-bark

Results of topical application of the stem-bark extracts on the *T. castaneum* strains given in tables 18-21. Pet. spt. extract of stem-bark, where LD_{50} values were ranging between 0.888 µg/µl (on CR-1 adult for 48 h) to 8.775 µg/µl (on CTC-12 adult for 48 h) and 0.946 µg/µl (on CR-1 pupae for 72 h) to 76.469 µg/µl (on FSS-II pupae for 24 h) (Table-18), then EtOAc extract of stem-bark, where LD_{50} values were ranging between 2.271 µg/µl (on FSS-II adult for 48 h) to 5.524 µg/µl (on CR-1 adult for 24 h) and 16.186 µg/µl (on CR-1 pupae for 72 h) to 161.641 µg/µl (on CTC-12 pupae for 24 h) (Table-19). Activity of EtOAc extracts were more or less similar to that of Pet. spt. extract efficacy. The lowest mortality was recorded in case of acetone extracts of the stem-bark, where LD_{50} values were ranging between 30735.710 µg/µl (on FSS-II pupae for 48 h) to 86.595 µg/µl (on CR-1 pupae for 72 h) (Table-20). Recovery was recorded in case of MeOH extract of the stem-bark for FSS-II adults for 48 to 72 hours of treatment and for CTC-12 24-, 48- to 72- hours of exposure (Table-21).

A triterpenoid **aphanamixinin** (Chandrasekharan *et al.*, 1968; Chatterjee *et al.*, 1970) from petrol extract, a **β-sitosterol** and a by Srivastava and Agnihotri (1984 and 1985), two saponins, **aglaiol-3-O-rhamnosyl-xyloside** from rectified spirit (Srivastava and Agnihotri, 1985) and **poriferasterol-3-O-rhamnoside** from EtOH extract (Agnihotri, 1987) two glycosides **1,5-dihydroxy-6,7,8-trimethoxy-2-methyl-anthraquinone-3-O-β-D-xylopyranoside** and **naringenin 7,4′-dimethyl ether-5-O-α-L-rhamnopyranoside** from the rectified spirit extract (Srivastava and Agnihotri, 1985) and a terpenoid **amoorinin** (Agnihotri *et al.*, 1987a,b) from EtOH extract and Srivastava and Agnihotri, (1985) reported **(24 R)-24-ethylcholestra-5, 22-diene-3-*O*-rhamnoside** but none on these findings were reported with mentioning their biological activities.

Table 18. Activity of Pet. spt. extract of *A. rohituka* stem-bark against *T. castaneum*.

Strain	Life stage	Exposure time (h)	LD_{50} value µg/µl	Regression equation	χ^2 value
CR-1	Pupae	24	3.729	y = 2.621 + 1.514x	1.013
		48	1.366	y = 3.508 + 1.314x	1.288
		72	0.946	y = 3.984 + 1.043x	0.590
	Adult	24	1.257	y = 3.523 + 1.343x	0.252
		48	0.888	y = 4.144 + 0.903x	1.478
		72	1.240	y = 3.481 + 1.389x	0.675
FSS-II	Pupae	24	76.469	y = 3.534 + 0.509x	0.124
		48	7.474	y = 4.097 + 0.482x	0.209
		72	3.974	y = 4.105 + 0.560x	0.451
	Adult	24	4.529	y = 4.314 + 0.414x	0.010
		48	2.480	y = 4.200 + 0.574x	0.087
		72	2.076	y = 4.154 + 0.642x	0.097
CTC-12	Pupae	24	7.427	y = 3.078 + 1.028x	0.510
		48	1.851	y = 3.269 + 1.366x	0.211
		72	1.725	y = 3.221 + 1.439x	0.306
	Adult	24	7.300	y = 4.323 + 0.363x	0.025
		48	8.775	y = 4.150 + 0.438x	0.007
		72	3.709	y = 4.139 + 0.548x	0.047

Table 19. Activity of EtOAc extract of *A. rohituka* stem-bark against *T. castaneum*.

Strain	Life stage	Exposure time (h)	LD_{50} value µg/µl	Regression equation	χ^2 value
CR-1	Pupae	24	79.764	y = 3.726 + 0.670x	0.011
		48	65.453	y = 3.952 + 0.577x	0.024
		72	16.186	y = 4.034 + 0.799x	0.283
	Adult	24	5.524	y = 4.230 + 1.038x	0.702
		48	4.534	y = 4.380 + 0.944x	0.252
		72	4.630	y = 4.106 + 1.344x	0.415
FSS-II	Pupae	24	78.292	y = 2.787 + 1.168x	0.166
		48	53.566	y = 3.361 + 0.948x	0.521
		72	33.727	y = 3.374 + 1.064x	0.509
	Adult	24	2.772	y = 4.332 + 1.509x	0.522
		48	2.271	y = 4.561 + 1.234x	0.633
		72	3.217	y = 4.112 + 1.750x	0.154
CTC-12	Pupae	24	161.641	y = 3.313 + 0.764x	0.099
		48	94.066	y = 3.492 + 0.764x	0.741
		72	32.691	y = 3.764 + 0.816x	1.448
	Adult	24	4.754	y = 4.483 + 0.763x	0.746
		48	4.754	y = 4.483 + 0.763x	0.746
		72	3.975	y = 4.562 + 0.731x	0.374

Table 20. Activity of acetone extract of *A. rohituka* stem-bark against *T. castaneum.*

Strain	Life stage	Exposure time (h)	LD_{50} value µg/µl	Regression equation	χ^2 value
CR-1	Pupae	24	1226.767	y = 1.965 + 0.983x	1.560
		48	111.026	y = 2.099 + 1.418x	0.679
		72	86.595	y = 3.558 + 0.744x	0.524
	Adult	24	236.4117	y = 2.035 + 1.249x	0.016
		48	253.495	y = 1.974 + 1.259x	0.219
		72	899.526	y = 3.025 + 0.669x	0.088
FSS-II	Pupae	24	-	-	-
		48	30735.710	y = 3.100 + 0.423	0.082
		72	9395.678	y = 3.032 + 0.495x	0.100
	Adult	24	2884.944	y = 3.034 + 0.568x	0.012
		48	655.038	y = 2.778 + 0.789x	1.600
		72	430.637	y = 3.025 + 0.750x	0.827
CTC-12	Pupae	24	11161.850	y = 2.739 + 0.559x	0.286
		48	615.378	y = 2.535 + 0.884x	0.521
		72	122.853	y = 2.450 + 1.221x	2.562
	Adult	24	994.281	y = 2.722 + 0.760x	0.477
		48	263.414	y = 1.928 + 1.269x	0.926
		72	152.085	y = 2.313 + 1.231x	1.107

Table 21. Activity of MeOH extract of *A. rohituka* stem-bark against *T. castaneum*.

Strain	Life stage	Exposure time (h)	LD_{50} value µg/µl	Regression equation	χ^2 value
CR-1	Pupae	24	4766.368	y = 3.147 + 0.504x	0.232
		48	948.231	y = 3.318 + 0.565x	0.784
		72	156.288	y = 3.263 + 0.792x	0.656
	Adult	24	1835.341	y = 3.103 + 0.581x	0.589
		48	1552.055	y = 3.479 + 0.477x	0.156
		72	637.312	y = 3.132 + 0.666x	0.063
FSS-II	Pupae	24	3906.750	y = 2,979 + 0.563x	0.005
		48	20941.310	y = 3.737 + 0.292x	0.663
		72	806.502	y = 3.141 + 0.640x	0.012
	Adult	24	1648.921	y = 3.732 + 0.394x	0.176
		48	728.092	y = 3.718 + 0.448x	0.079
		72	720.077	y = 3.532 + 0.514x	0.050
CTC-12	Pupae	24	258.628	y = 1.319 + 1.526x	0.401
		48	451.052	y = 2.024 + 1.121x	0.122
		72	3339.128	y = 3.254 + 0.496x	0.017
	Adult	24	1347.505	y = 3.289 + 0.547x	0.255
		48	11604.330	y = 3.312 + 0.415x	0.102
		72	836.176	y = 2.658 + 0.801x	0.577

3.2.5. Activity of the extracts from the roots

Results for the effects of root extracts collected in Pet. spt., EtOAc, acetone and MeOH. on *T. castaneum* strains are given in tables 22-25. In the present investigation highest mortality was recorded in case of the Pet. spt. extract of the roots, where LD_{50} values were ranging between 0.657 µg/µl (on CR-1 pupae for 24 h) to 2.156 µg/µl (on FSS-II pupae for 72 h) and 1.897 µg/µl (on CR-1 adult for 24 h) to 7.271 µg/µl (on FSS-II adult for 48 h) (Table 22),), then EtOAc extract of roots, where LD_{50} values were ranging between 0.597 µg/µl (on CTC-12 adult for 48 h) to 20.690 µg/µl (on FSS-II adult for 24 h) and 3.462 µg/µl (on CR-1 pupae for 72 h) to 8.925 µg/µl (on FSS-II pupae for 24 h) (Table-23), then acetone extract of roots, where LD_{50} values were ranging between 8.841 µg/µl (on CR-1 pupae for 72 h) to 293.064 µg/µl (on FSS-II pupae for 24 h) and 6.117 µg/µl (on CR-1 adult for 72 h) to 63.590 µg/µl (on CR-1 adult for 24 h) (Table-24). The lowest mortality was observed in case of MeOH extracts of roots, where LD_{50} values were ranging between 34708.590 µg/µl (on FSS-II pupae for 24 h) to 165.147 µg/µl (on FSS-II pupae for 72 h) (Table-25). In case of EtOAc extracts the mortality was higher in adults, but in case of acetone and MeOH extracts it created an equilibrium in mortality in both the adults and pupae. The activity of Pet. spt. extract is significant enough because of comparatively high mortality and of recovery found in case of all CR-1, FSS-II and CTC-12 pupae and CR-1 and FSS-II adults. Recovery was recorded for all possible cases which could be traced out from the fluctuation or increased mode of the LD_{50} values (Table-22).

From MeOH extract of roots a flavone glycoside **8-C-methyl quercetin-3-O-β-D-xylopyranoside** (Jain and Srivastava, 1985) and, a saponin **Betulin-3-*β*-*O*-*β*-D-xylopyranoside** (Jain and Srivastava, 1984) were reported without mentioning there biological activity.

Table 22. Activity of Pet. spt. extract of *A. rohituka* root against *T. castaneum*.

Strain	Life stage	Exposure time (h)	LD_{50} value µg/µl	Regression equation	χ^2 value
CR-1	Pupae	24	0.657	y = 4.359 + 0.783x	0.323
		48	0.716	y = 4.311 + 0.806x	0.093
		72	0.670	y = 4.508 + 0.595x	0.0002
	Adult	24	1.897	y = 3.524 + 1.155x	0.108
		48	2.193	y = 3.424 + 1.176x	0.315
		72	3.012	y = 3.187 + 1.226x	0.050
FSS-II	Pupae	24	1.751	y = 3.376 + 1.306x	0.232
		48	2.015	y = 3.434 + 1.201x	0.138
		72	2.156	y = 3.659 + 1.006x	0.345
	Adult	24	3.240	y = 3.972 + 0.680x	0.058
		48	7.271	y = 3.918 + 0.581x	0.422
		72	3.256	y = 2.948 + 1.356x	1.127
CTC-12	Pupae	24	0.718	y = 4.559 + 0.516x	0.928
		48	0.844	y = 4.642 + 0.387x	0.174
		72	1.831	y = 4.585 + 0.329x	0.085
	Adult	24	6.488	y = 3.638 + 0.752x	0.161
		48	6.518	y = 3.754 + 0.687x	0.327
		72	5.729	y = 3.429 + 0.894x	0.403

Table 23. Activity of EtOAc extract of *A. rohituka* root against *T. castaneum*.

Strain	Life stage	Exposure time (h)	LD_{50} value µg/µl	Regression equation	χ^2 value
CR-I	Pupae	24	7.690	y = 4.647 + 0.398x	0.070
		48	3.553	y = 4.729 + 0.492x	0.327
		72	3.462	y = 4.588 + 0.764x	0.010
	Adult	24	2.506	y = 4.730 + 0.676x	0.105
		48	2.388	y = 4.730 + 0.713x	0.483
		72	2.195	y = 4.569 +1.263x	0.489
FSS-II	Pupae	24	8.925	y = 4.544 + 0.480x	0.027
		48	7.402	y = 4.473 + 0.606x	0.594
		72	5.126	y = 4.468 + 0.749x	0.030
	Adult	24	20.690	y = 4.424 + 0.438x	0.007
		48	5.809	y = 4.284 + 0.937x	1.286
		72	3.961	y = 4.291 + 1.186x	0.365
CTC-12	Pupae	24	4.437	y = 4.606 + 0.608x	0.144
		48	5.730	y = 4.484 + 0.680x	0.160
		72	6.942	y = 4.523 + 0.566x	0.097
	Adult	24	9.966	y = 5.251 + 0.251x	0.107
		48	0.597	y = 5.086 + 0.385x	0.121
		72	1.097	y = 4.977 + 0.571x	0.065

Table 24. Activity of acetone extract of *A. rohituka* root against *T. castaneum*.

Strain	Life stage	Exposure time (h)	LD_{50} value µg/µl	Regression equation	χ^2 value
CR-1	Pupae	24	59.562	y = 2.353 + 0.954x	0.068
		48	12.962	y = 2.265 + 1.294x	0.769
		72	8.841	y = 1.905 + 1.590x	0.190
	Adult	24	63.598	y = 2.295 + 0.965x	0.447
		48	22.486	y = 2.300 + 1.148x	0.373
		72	6.117	y = 2.643 + 1.319x	1.574
FSS-II	Pupae	24	293.064	y = 3.049 + 0.563x	0.005
		48	42.678	y = 3.271 + 0.658x	0.158
		72	9.411	y = 2.258 + 1.390	0.180
	Adult	24	50.329	y = 3.082 + 0.710x	0.674
		48	28.497	y = 3.037 + 0.800x	0.498
		72	9.337	y = 2.744 + 1.145x	0.211
CTC-12	Pupae	24	49.937	y = 2.763 + 0.829x	0.194
		48	67.095	y = 2.838 + 0.765x	0.024
		72	10.100	y = 2.601 + 1.197x	0.900
	Adult	24	36.091	y = 3.030 + 0.770x	0.042
		48	40.022	y = 3.781 + 0.468x	0.726
		72	10.655	y = 3.611 + 0.685x	0.179

Table 25. Activity of MeOH extract of *A. rohituka* root against *T. castaneum*.

Strain	Life stage	Exposure time (h)	LD_{50} value µg/µl	Regression equation	χ^2 value
CR-I	Pupae	24	7135.857	y = 2.831 + 0.563x	0.005
		48	541.775	y = 3.011 + 0.728x	0.078
		72	295.097	y = 2.386 + 1.058x	0.047
	Adult	24	858.918	y = 2.186 + 0.959x	0.262
		48	468.937	y = 1.946 + 1.143x	0.225
		72	565.188	y = 2.452 + 0.926x	0.295
FSS-II	Pupae	24	34708.590	y = 3.385 + 0.356x	0.068
		48	2310.786	y = 2.963 + 0.606x	0.041
		72	165.147	y = 3.208 + 0.808x	0.063
	Adult	24	7971.730	y = 3.377 + 0.416x	0.023
		48	820.670	y = 3.146 + 0.636x	0.200
		72	309.346	y = 3.297 + 0.684x	0.431
CTC-12	Pupae	24	2520.053	y = 3.114 + 0.554x	0.210
		48	1075.570	y = 2.605 + 0.790x	0.155
		72	355.933	y = 2.825 + 0.853x	0.014
	Adult	24	1449.955	y = 1.985 + 0.954x	0.068
		48	1451.680	y = 2.093 + 0.919x	0.004
		72	849.3481.	y = 2.074 + 0.999x	0.939

3.2.6. Summary discussion on insecticidal tests

Results of topical application of the crude extracts in different concentration in the dose-mortality experiments were less satisfactory, while test on three standard strains of the red flour beetle *T. castaneum* offered some predictions of the locations of biologically active constituents in the title plant *A. rohituka*. The results were not so clear on the differences on the resistant variety CTC-12 than to more susceptible variety FSS-II, even though, fair attention paid on this subject in repetition. To make the findings more conspicuous and to make the concepts more furnished it was felt necessary to conduct other biological tests for other projections concerning various other activities of the plant extracts. For the evaluation of the constituents for pesticidal or medicinal properties it needs tests for different directions, i.e., for pests: test against insects, molluscs, nematodes, rodents, etc. and on different forms of their life stages; for medicine: test against pathogenic organisms, vectors, etc. In these perspectives some other bioassays have been selected.

3.3. Other bio-autographic assessments

Evaluation of the extractives of *A. rohituka* has been made by a series of bioassays with human pathogenic bacteria *B. subtilis*, human pathogenic fungi *C. albicans*, Plant pathogenic fungi *C. cucumerinum*, stored grain insect pest *T. castaneum*, mosquito vector of the yellow fever *A. aegypti* larvae and schistosomiasis transmitting snails *B. glabrata* and it was found that different parts of this plant, viz. leaves, seeds, fruit-pericarp, stem-bark and roots bearing biologically active constituents. A checklist of the bioassays carried out on the extractives of the experimental plant given in the table 26.

Table 26. Bioassay checklist of *A. rohituka* extracts.

Organs	Extracts	Test agents				
		Aedes aegypti	*Biomphalaria galabrata*	*Cladosporium cucumerinum*	*Candida albicans*	*Bacillus subtilis*
Leaf	Pet. spt.	+	++/ 400 ppm/24h	+	+	+
	EtOAc	+	++/ 400 ppm/24h	+	+	+
	acetone	+	++/400 ppm/24h	+	+	+
	MeOH	-	-	-	+	+
Seed	Pet. spt.	-	-	-	+	-
	EtOAc	-	-	-	+	-
	acetone	-	-	-	-	-
	MeOH	-	-	-	-	-
Fruit pericarp	Pet. spt.	+	-	-	+	-
	EtOAc	+	-	-	-	-
	acetone	+	-	-	-	-
	MeOH	-	-	-	-	-
Stem-bark	Pet. spt.	-	-	-	-	-
	EtOAc	-	-	-	-	-
	acetone	-	++/400-200-100ppm/24h	-	+	+
	MeOH	+	++/400ppm/24h	+	-	+
Root	Pet. spt.	-	++/400 ppm/24h	+	+	+
	EtOAc	-	++/400 ppm/24h	-	-	-
	acetone	-	++/400 ppm/24h	-	+	-
	MeOH	*	*	*	*	*

+ = active, (-) = not active and * = sample was not available.

3.3.1. Activity against *A. aegypti* larvae

Leaf extracts collected in Pet. spt., EtOAc and acetone did not show activity within 30 min. and offered mortality of 26.67%, 50% and 40% respectively after 12 h of exposure. Those were also active in a lower concentration of 250 µg/ml and the mortality recorded after 12 h of exposure was 20%, 26.67% and 23.33%, after 24 h of exposure, 66.67%, 50% and 60%, and after 36 h of exposure, 86.67%, 63.33% and 90% for the extracts collected in Pet. spt., EtOAc and acetone respectively.

The seed extracts, each with a concentration of 500 µg/ml in fresh-water offered no activity within 30 min. for Pet. spt., acetone and MeOH. With a double concentration it showed activity for Pet. spt., acetone and MeOH extracts after 12 h of exposure and the mortality found 6.67%, 33.33% and 6.67%; after 24 h of exposure 20%, 10% and 16.67% and after 36 h of exposure 23.33%, 16.67% and 13.33% respectively.

The fruit-pericarp extracts, each with a concentration of 500 µg/ml in fresh-water offered exciting results showed activity within 30 min. for Pet. spt., EtOAc, acetone extract. After 12 h of exposure the mortality were counted 100%, 66.67%, 76.67% and 50% respectively. The concentration then diluted into 250 µg/ml for all four Pet. spt., EtOAc, acetone and MeOH extracts and the respective mortality observed after 12 h of exposure 26%, 20%, 20% and 10%, and after 24 h of exposure 40%, 40% 33.33% and 43.33% and after 36 h of exposure 60%, 90%, 70% and 70% respectively.

No mortality was found after 30 min. of exposure in case of stem-bark extract. Methanol extract of stem-bark offered mortality after 12 h of exposure 70%, after 24 h 80% and after 36 h 83.33%. With a double concentration it showed activity for Pet. spt., EtOAc, acetone and MeOH extracts after 12 h of exposure and mortality found 33.33%,

23.33%, 26.33% and 73.33%; after 24 h of exposure 43.33%, 33.33%, 36.67% and 80% and after 36 h 73.33%, 66.67% 60% and 93.33% respectively.

Root extracts showed no mortality even for a double concentration after 30 min. of exposure except Pet. spt. extract. The mortality was 10% after 12 h of exposure for Pet. spt., EtOAc and acetone extracts, which were 20%, 30% and 26.67% after 24 h and 36.67%, 36.67% and 46.67% after 36 h of exposure (Table 27).

3.3.2. Activity against *B. glabrata*

Leaf extracts collected in Pet. spt., EtOAc and acetone; stem-bark extract collected in acetone and MeOH; and root extracts in Pet. spt., EtOAc and acetone, offered mortality to *B. glabrata* with a concentration of 400 ppm in water after 24 h of exposure.

No mortality was found in case of MeOH extract of leaves, Pt. spt., EtOAc, acetone and MeOH extracts of seeds and fruit-pericarp, Pet. spt and EtOAc extract of stem-bark. On the other hand, acetone extract of stem bark offered mortality even in a lower concentration (100 ppm) after 24 h of exposure (Table 28).

Table 27. Activity of *A. rohituka* extracts against *A. aegypti* larvae.

Solvents used	Sourcr materials																			
	Leaf@				Seeds$				Fruit-pericarp@				Stem-bark$				Roots$			
	+	A	B	C	+	A	B	C	+	A	B	C	+	A	B	C	+	A	B	C
Pet. spt	n	6	20	26	n	2	6	7	y	8	12	18	n	10	13	22	n	3	6	11
EtOAc	n	8	15	19	*	*	*	*	y	6	12	27	n	7	10	20	n	3	9	11
acetone	n	7	18	27	n	1	3	5	y	6	10	21	n	7	11	18	n	3	8	14
MeOH	n	0	0	0	n	2	5	4	n	3	13	21	n	22	24	28	*	*	*	*

+ = activity after 30 min.;

A, B and C= mortality after 12 -, 24- and 36 hours of exposure;

y= active; n = not active; 1,2,6,12 etc. = # killed; @ =1/2 dose; $= double dose; *= sample not available.

The result discussed here justified changing doses to provide mortality in all available samples and doses were lessen @, in case of leaf and fruit pericarp, and doubled $ in all other cases.

Table 28. Activity of *A. rohituka* extracts against *B. glabrata.*

Solvents used	**Source materials**				
	Leaf	Seed	Fruit-pericarp	Stem-bark	Root
Pet. spt.	++/400ppm/24h	-00-	-00-	-00-	++/400ppm/24h
EtOAc	++/400ppm/24h	-00-	-00-	-00-	++/400ppm/24h
acetone	++/400ppm/24h	-00-	-00-	++/400-200-100 ppm/24h	++/400ppm/24h
MeOH	-00-	-00-	-00-	++/400ppm/24h	*

-00- = not active;

* = sample not available;

++ = active for both of the snails.

3.3.3. Activity against *C. cucumerinum*

For the standard amount of 100 µg/spot of the crude extracts run on TLC plates up to 8 cm which showed inhibition zones for the leaf extract in Pt. spt. with Rf values 0.25; in EtOAc, 0.09, 0.17 and 0.38 (of which the 2nd and the 3rd one from the base is more distinct), in acetone 0.13, for stem-bark extract collected in EtOAc 0.08, root extract in Pet. spt. 0.19 (very weak) and no activity found in case of all MeOH extracts (Table 29).

3.3.4. Activity against *C. albicans*

For *C. albicans* most of the crude extracts found active for the standard amount of 100 µg/spot on the TLC, while the inhibition zones for leaf extracts in Pet. spt. with Rf values 0.19; in EtOAc 0.19, 0.38, 0.53 and 0.63, in acetone 0.19, 0.28 and 0.53; for seed extract in Pet. spt. 0.06, 0.19 and 0.28, in EtOAc 0.19; in acetone 0.28; fruit-pericarp extract in Pet. spt. 0.09 and 0.28; stem-bark extract in Pet. spt. 0.19 and 0.31; in acetone 0.31; and root extract in Pet. spt. 0.22, and 0.41; in acetone at 0.19 and leaf, seed, fruit-pericarp extract in MeOH showed inhibition zones all at 0.94, while root extract in MeOH at zero (Table 30).

3.3.5. Activity against *B. subtilis*

Leaf extracts in Pet. spt., EtOAc, acetone and MeOH; seed extracts in Pet. spt., EtOAc and acetone, fruit-pericarp extract in Pet. spt., EtOAc and acetone and root extracts with Pet. spt. found active. The standard sample amount is 100 µg/spot. The samples spotted on the TLC plates run almost 8 cm and the active constituents showed inhibition zones for leaf extracts collected in Pet. spt. with Rf values 0.13, 0.25 and 0.50; in EtOAc 0.25, 0.44, 0.50 and 0.63; in acetone 0.19, 0.25 and 0.50; in MeOH 0.84; stem-bark extract in acetone at zero; root extract in Pt. spt. 0.19 (Table 31), while the seed and fruit-pericarp extracts in Pet. spt., EtOAc and acetone showed very weak activities but were negligible, while seed and fruit-pericarp extract in MeOH were not active at all.

Table 29. Activity of *A. rohituka* extracts against *C. cucumerinum*.

Solvents used	Source materials				
	Leaf	Seed	Fruit-pericarp	Stem-bark	Root
Pet. spt.	y	n	n	n	n
EtOAc	y	n	n	y	n
acetone	y	n	n	n	n
MeOH	n	n	n	*	n

y= active, n = not active and *= sample not available.

Table 30. Activity of *A. rohituka* extracts against *C. albicans*.

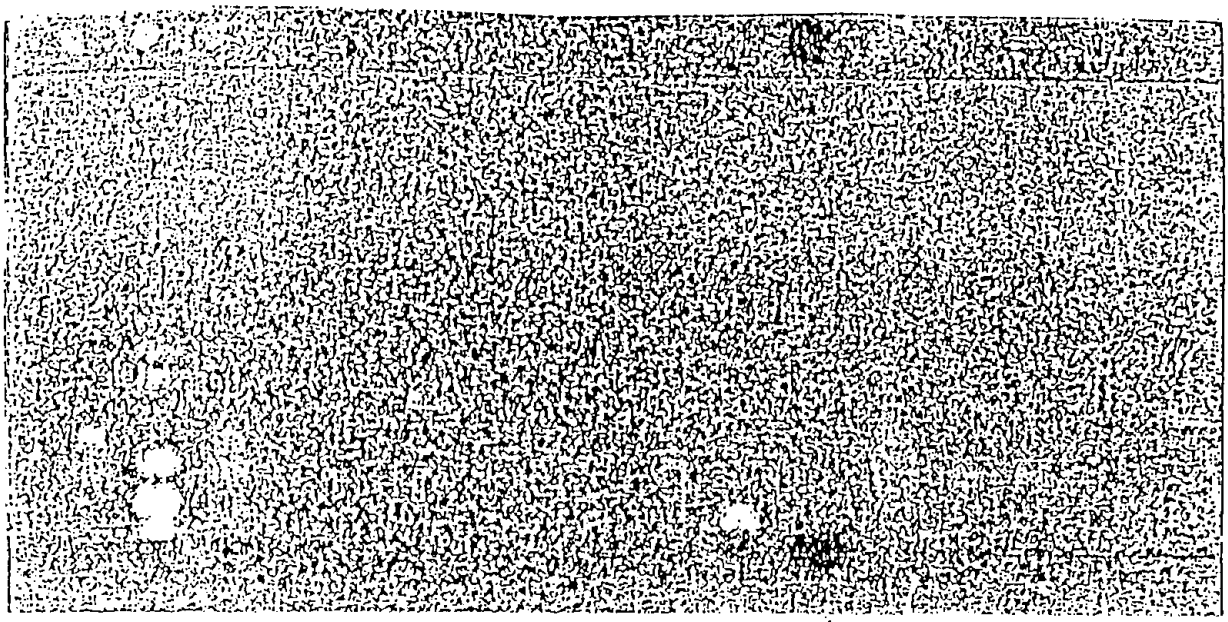
Solvents used	Source materials				
	Leaf	Seed	Fruit-pericarp	Stem-bark	Root
Pet. spt.	y	y	y	y	y
EtOAc	y	y	n	n	n
acetone	y	y	n	y	y
MeOH	y	y	y	*	y

y= active, n = not active and *= sample not available.

Table 31. Activity of *A. rohituka* extracts against *B. subtilis*.

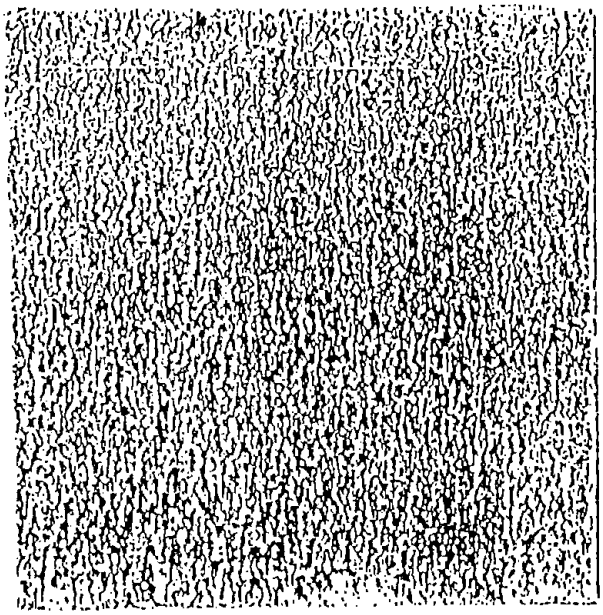
Solvents used	Source materials				
	Leaf	Seed	Fruit-pericarp	Stem-bark	Root
Pet. spt.	y	n	n	n	y
EtOAc	y	n	n	n	n
acetone	y	n	n	y	n
MeOH	y	n	n	*	n

y= active, n = not active and *= sample not available.



LP LE LA SP SE SA ScP ScA BP BE BA RP RE RA

Test with Pet. spt., EtOAc and acetone extracts



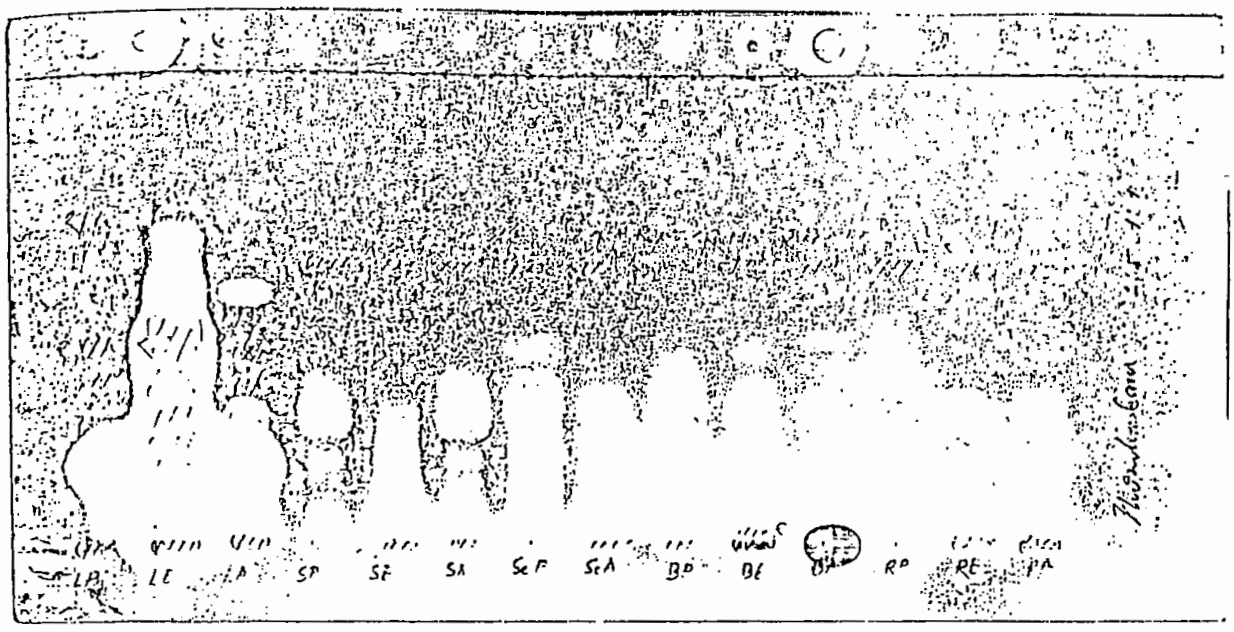
LM SM ScM RM

Test with MeOH extracts

L = Leaf
S = Seeds
Sc = Seed-coat (fruit-pericarp)
B = Bark (stem-bark)
R = Roots

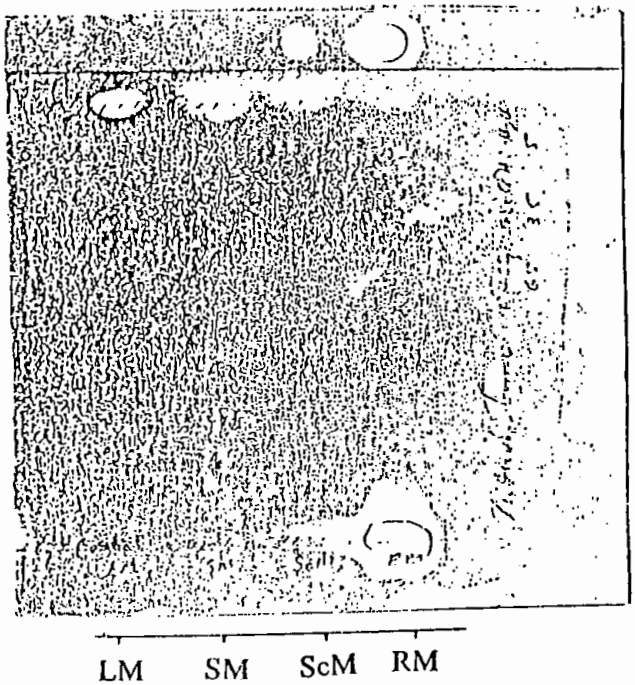
P = Extracted in Pet. spt.
E = Extracted in EtOAc
A = Extracted in acetone
M = Extracted in MeOH

Fig. 14. Activity of *A. rohituka* extracts against *C. cucumerinum*.



LP LE LA SP SE SA ScP ScA BP BE BA RP RE RA

Test with Pet. spt., EtOAc and acetone extracts



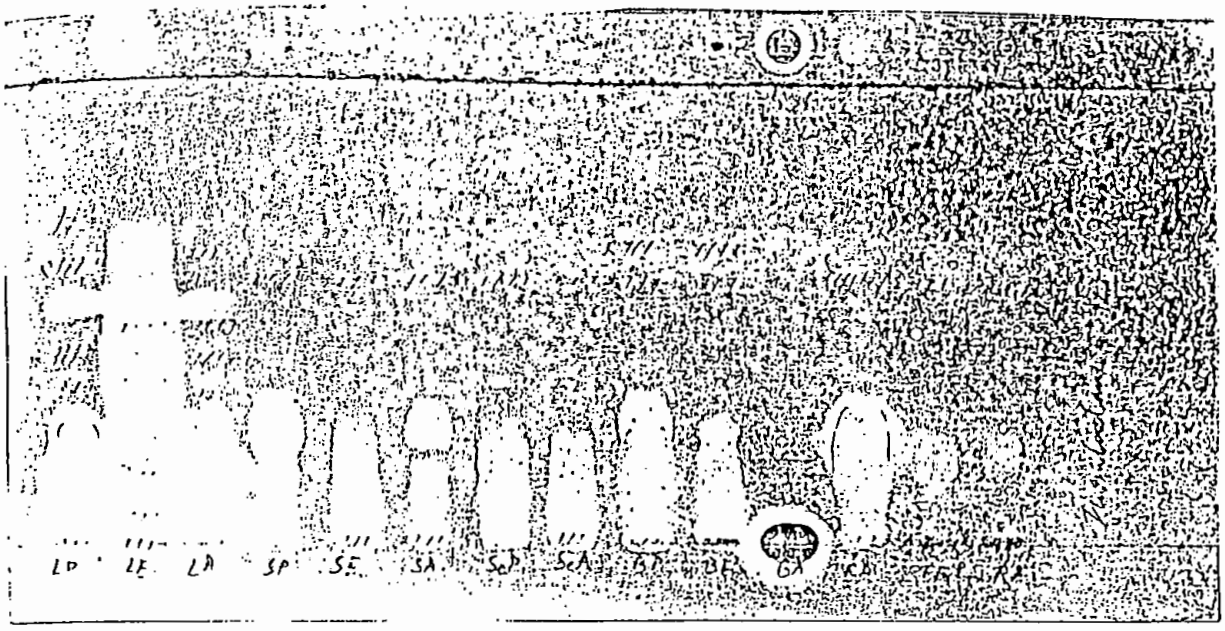
LM SM ScM RM

Test with MeOH extracts

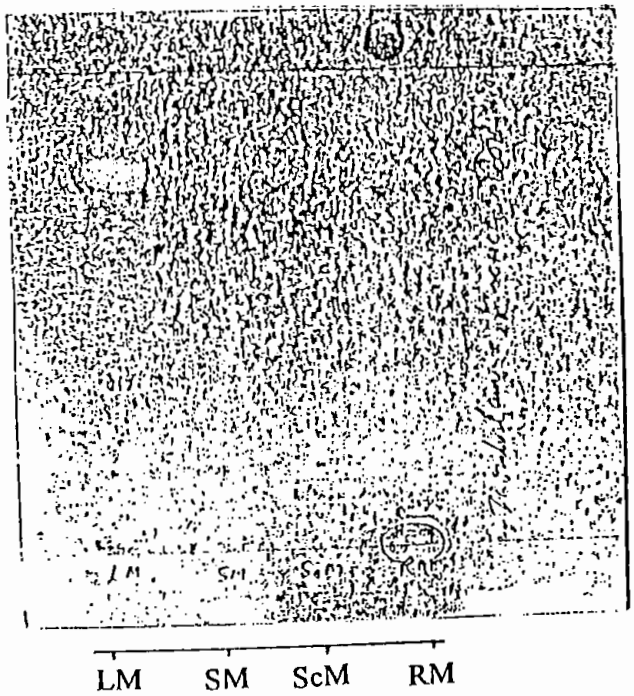
L = Leaf
S = Seeds
Sc = Seed-coat (fruit-pericarp)
B = Bark (stem-bark)
R = Roots

P = Extracted in Pet. spt.
E = Extracted in EtOAc
A = Extracted in acetone
M = Extracted in MeOH

Fig. 15. Activity of *A. rohituka* extracts against *C. albicans*.



Test with Pet. spt., EtOAc and acetone extracts



Test with MeOH extracts

L = Leaf
S = Seeds
Sc = Seed-coat (fruit-pericarp)
B = Bark (stem-bark)
R = Roots

P = Extracted in Pet. spt.
E = Extracted in EtOAc
A = Extracted in acetone
M = Extracted in MeOH

Fig. 16. Activity of *A. rohituka* extracts against *B. subtilis*.

3.3.6. Conclusion and perspectives of bioassays

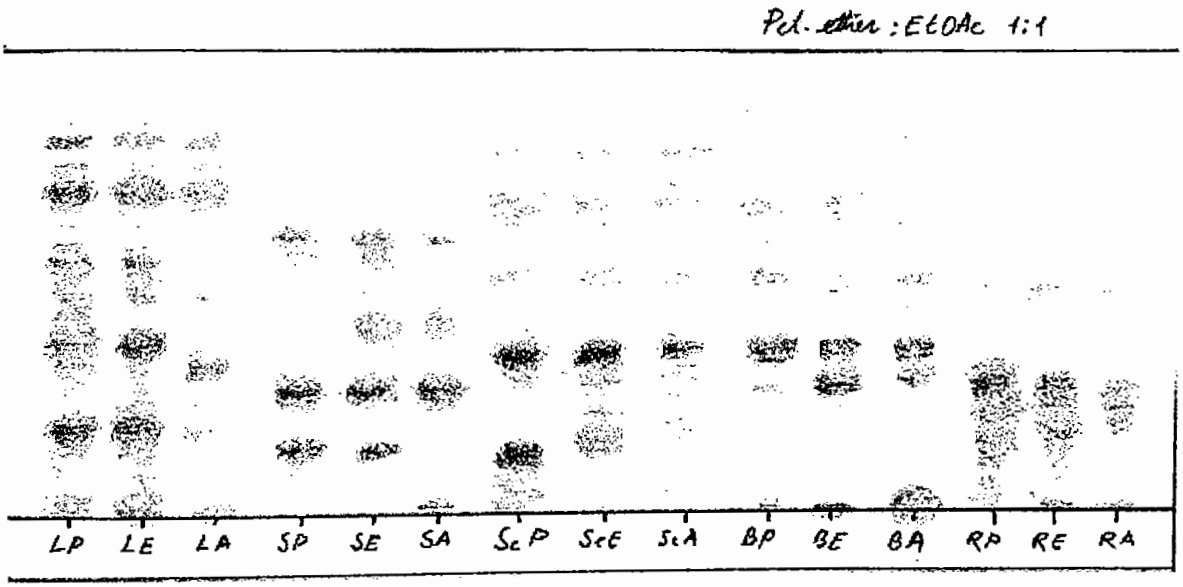
From all these experiments regarding bioassays mentioned above it is found that most of the extracts collected in four different solvents are active against the bacterium *B. subtilis*, fungi *C. cucumerinum*, and *C. albicans*, insect pest *T. castaneum*, mosquito larvae *A. aegypti* and the snails *B. glabrata*. Much clear inhibition zones were found in case of EtOAc extract of leaves against *B. subtilis*, *C. cucumerinum*. and *C. albicans* and it was also showed satisfactory activity against the mosquito larvae and the snails.

Moreover, in comparison to stem-bark and roots, leaves are studied less. And that is why leaves were taken for this study. As in screening EtOAc extract of *A. rohituka* leaves was found active on all target organisms, and it was subjected to further investigation by activity guided fractionation. Activity against *C. cucumerinum* was taken as a tool experiment for this fractionation with a view to isolate, purify and characterize antifungal properties that afforded two active compounds.

3.4. Chromatographic information

3.4.1. Chromatography on TLC

For the normal phase chromatography Silica-gel Si60 F254 on glass and Al sheets (Merck) were used. Ten mg/ml of the sample in the solvent of extract offered 100 µg/spot while spotted 10 µl for each of the samples. The chromatograms then developed within a conventional chamber (Camag) with the solvent systems: for Pet. spt., EtOAc and acetone extracts- Pet. ether:EtOAc (1:1); and for MeOH extract CHCl3:MeOH:H2O (65:35:5); and all chromatograms were observed under UV at 254 and 366 nm and marked with a pencil. After complete run, the plates sprayed with the Godin reagent and on the dried TLC plate 10% ethanolic solution of H2SO4 is spayed before drying the plate at 100°C to reveal the spots of the compounds (Fig. 17).



L = Leaf, S = Seeds, Sc = Seed-coat (Fruit-pericarp), B = Bark (Stem-bark), R = Roots,
P = Extracted in Pet. spt., E = Extracted in EtOAc, A = Extracted in acetone and M = Extracted in MeOH

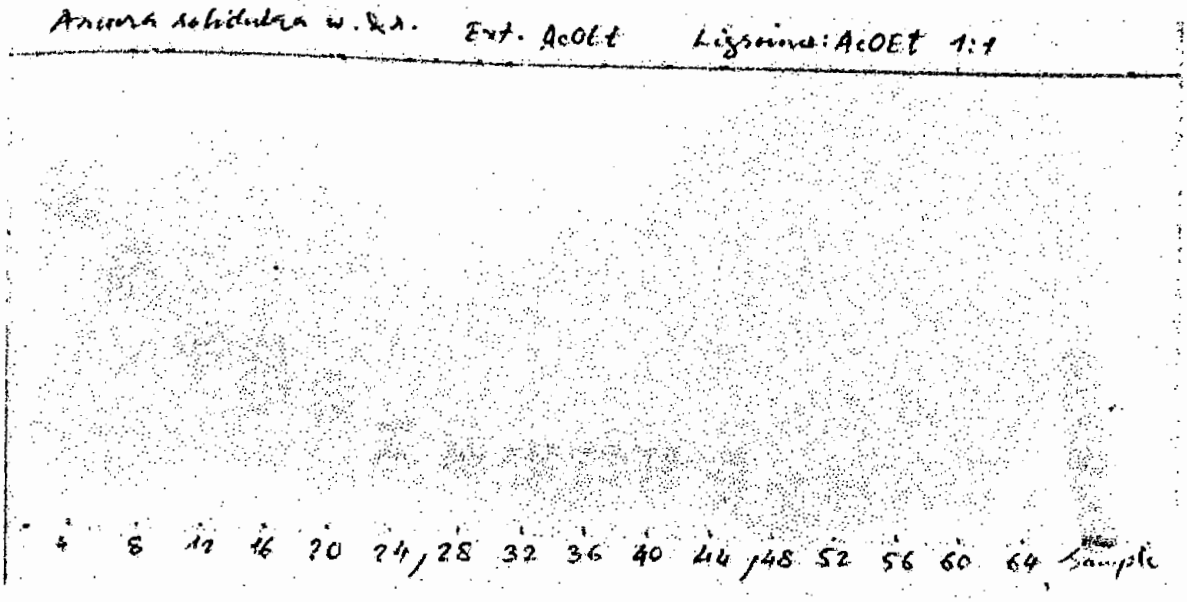
Fig. 17. Revelation of the compounds of the *A. rohituka* extracts.

3.4.1.1 Output by bioassay guided fractionation

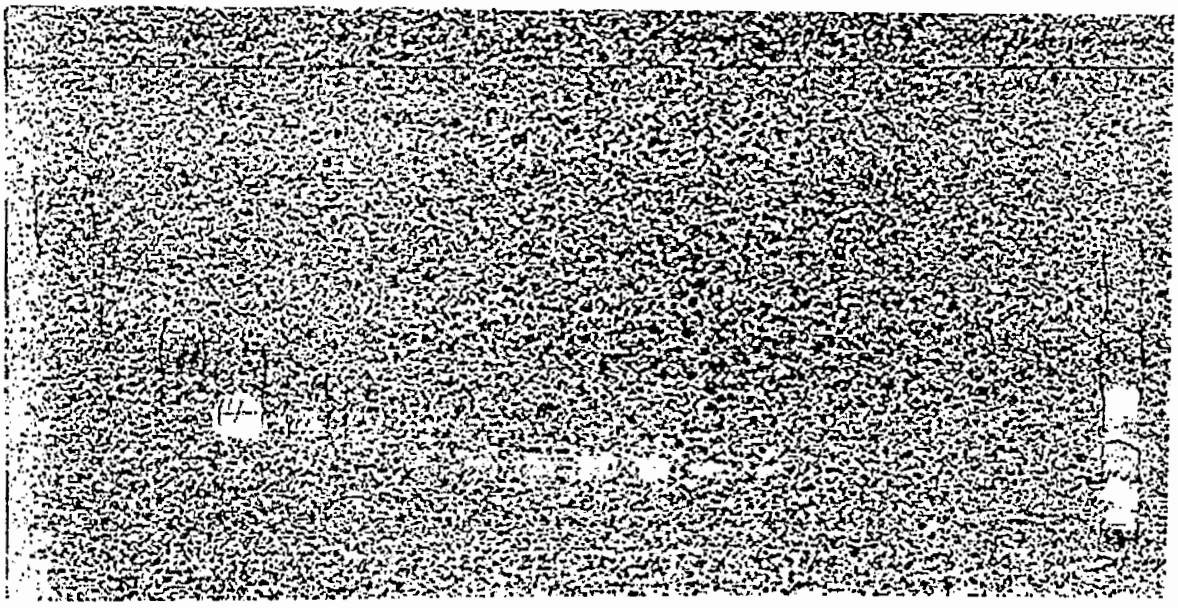
The fractions received from the first step of isolation were spotted on a TLC plate to reveal with Godin reagent for the fractions with a major spot of pink-violet in colour as the batch 'A' and another group of fractions for another major spot, violet in colour, as the batch 'B'. The fractions were also tested against *C. cucumerinum* to be sure about their activity on the test organism. They were observed on the UV light at 254 and 366 nm and the nature of their colour revelation with Godin also observed. The fractions 14-20 for 'A' and 28-43 for 'B' were found active against *C. cucumerinum* (Fig.18) and 'A' was visible as blackish spot under UV at 254 nm while 'B' was not active for both the wave-lengths.

3.4.1.2. Activity comparison test of the purified compounds

To compare the activity of the isolated compounds tests were carried out with a serial dilution method against *C. cucumerinum, C. albicans* and *B. subtilis*. Both the compounds were spotted on the same plate in each of the steps. For the test against *C, cucumerinum* the spots contained 30-, 20-, 10-, 5- and 1- µg of the products, and the compound 1 was found active until the last concentration while the another one was found not active (Fig. 19). The solvent system used to run the TLC was $CHCl_3$:MeOH:H_2O; (85:15:01). Other tests were done against *C. albicans* and *B. subtilis* with a concentration of 1 and 5 µg and were found active in both the cases (Fig. 20) while the solvent system used to run the TLC was $CHCl_3$:MeOH:H_2O; (97:03:01).



Revelation of the fractions with Godin reagent



Test of the same fractions with *C. cucumerinum.*

Fig. 18. Activity guided fractionation by TLC.

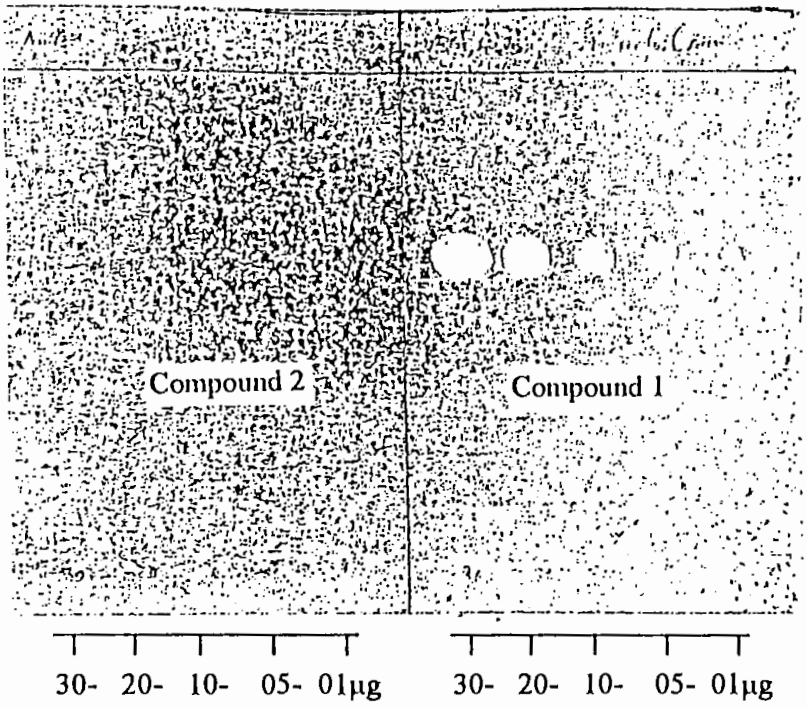


Fig. 19. Activity of the found compounds on *C. cucumerinum*.

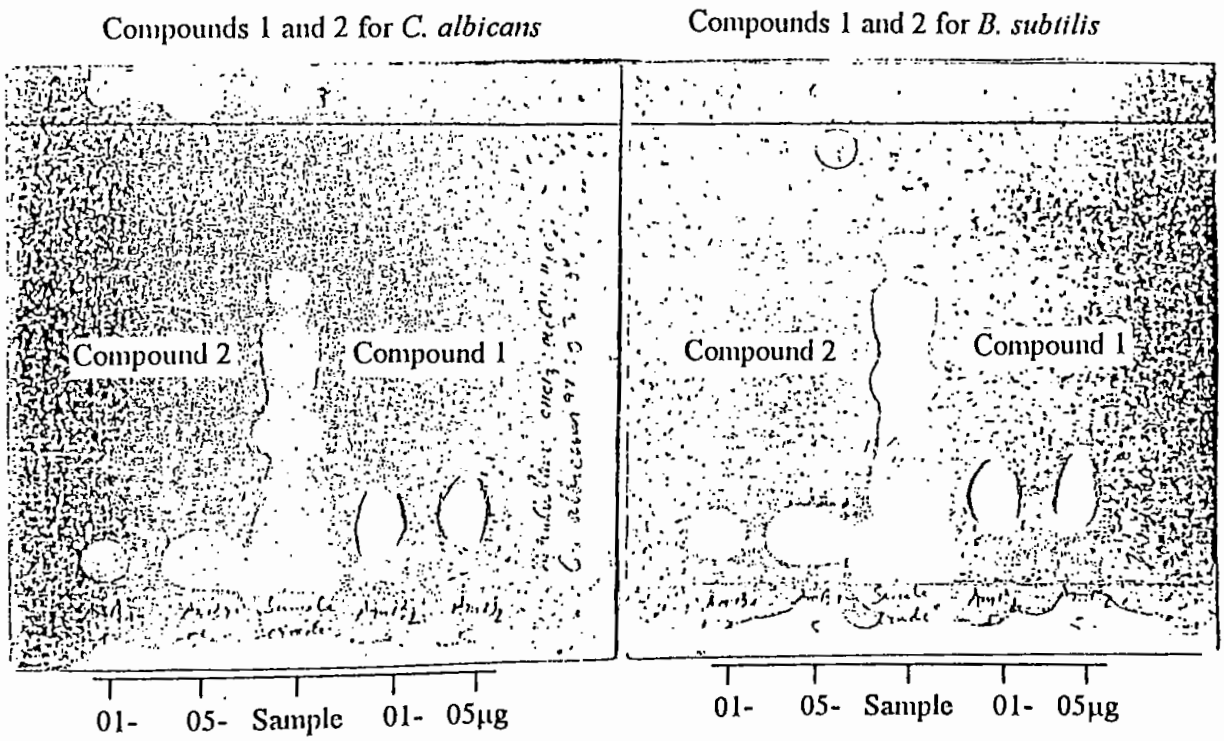


Fig. 20. Activity of the found compounds on *C. albicans* and *B. subtilis*.

3.4.2. High performance liquid chromatography

For the analytic HPLC the equipment used, were pump- 'Spectra physics 8700' detector- 'HP1090' (Hewlett Packard) and columns- 'Novapak' to give the probable resonance time of the compounds, and to furnish the purity of the isolated compounds as well (Fig. 21, 22).

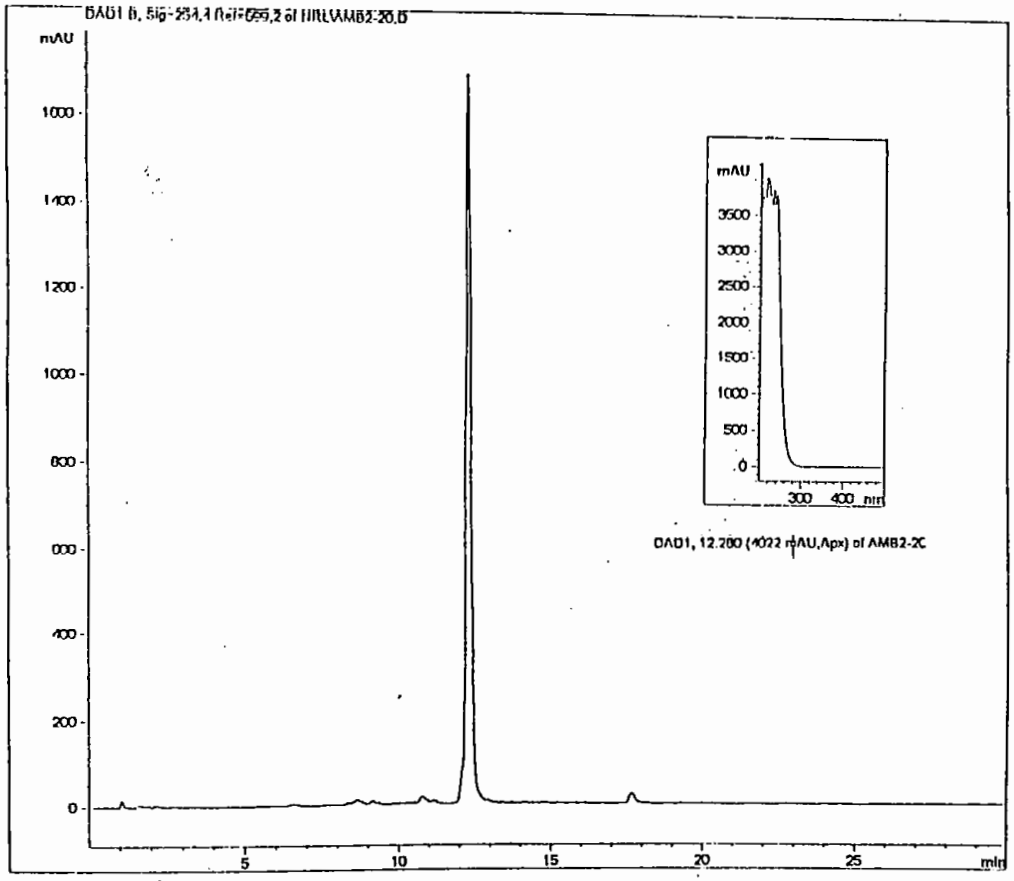


Fig. 21. HPLC spectrum of the compound 1.

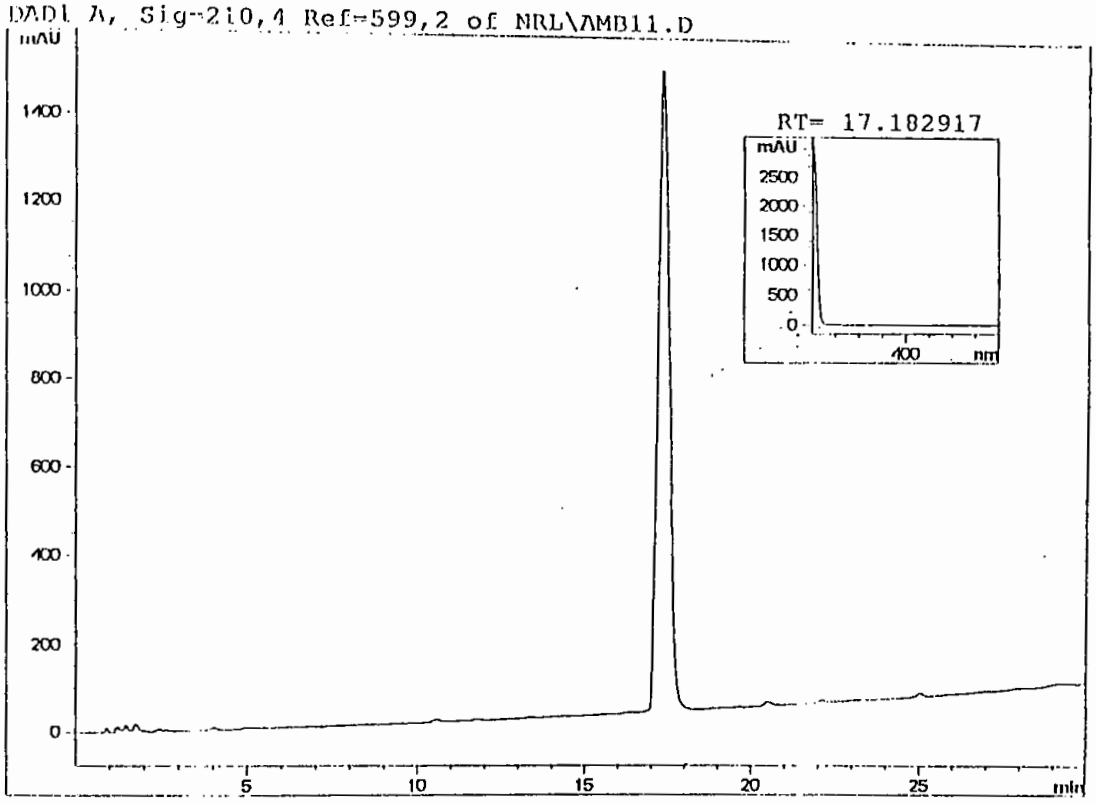


Fig. 22. HPLC spectrum of the compound 2.

3.5. Physiochemical methods

3.5.1. Infra red spectrometry

To prepare the sample for IR spectroscopy 1.5 mg of the **compound 2** was mixed well with 70 mg of Kbr in a mortar and pestle and a film was prepared using screw devices as a tool. Infrared spectroscopy was done only for the **compound 2,** which revealed the presence of a -OH group showing a band at 3310 cm^{-1} and tertiary 6-methyl at 1449 cm^{-1} and gem-dimethyl group at 1378 cm^{-1} (Fig. 23).

3.5.2. Mass spectrometry

The samples were prepared with a concentration of less than 10 mg/ml in HPLC grade MeOH (Fluka) and $CHCL_3$ (CD_3). From the mass spectrum the compound offered a molecular ion peak appeared at M/z 239 (M^+) to give the molecular weight of the compound is 238 which corresponds with the molecular formula $C_{14}H_{22}O_3$ (Fig. 24), and for the **compound 2** a molecular ion peak at M/z 308 (M^+). So, the molecular weight of the **compound** 2 is 308 which corresponds with the molecular formula $C_{20}H_{36}O_2$ ($[M]^+$ M/z 308) (Fig. 25).

3.5.3. Nuclear Magnetic Resonance of ^1H- protons and ^{13}C atoms

More than 7 mg of the **compound 1** and 35 mg of the **compound 2** prepared separately in HPLC grade MeOH and $CHCl_3$ because of super solubility in these solvents. A trace amount of TMS [(Tetramethylsilane, $(CD_3)_4$ Si)] was added as reference (Fig. 26, 27).

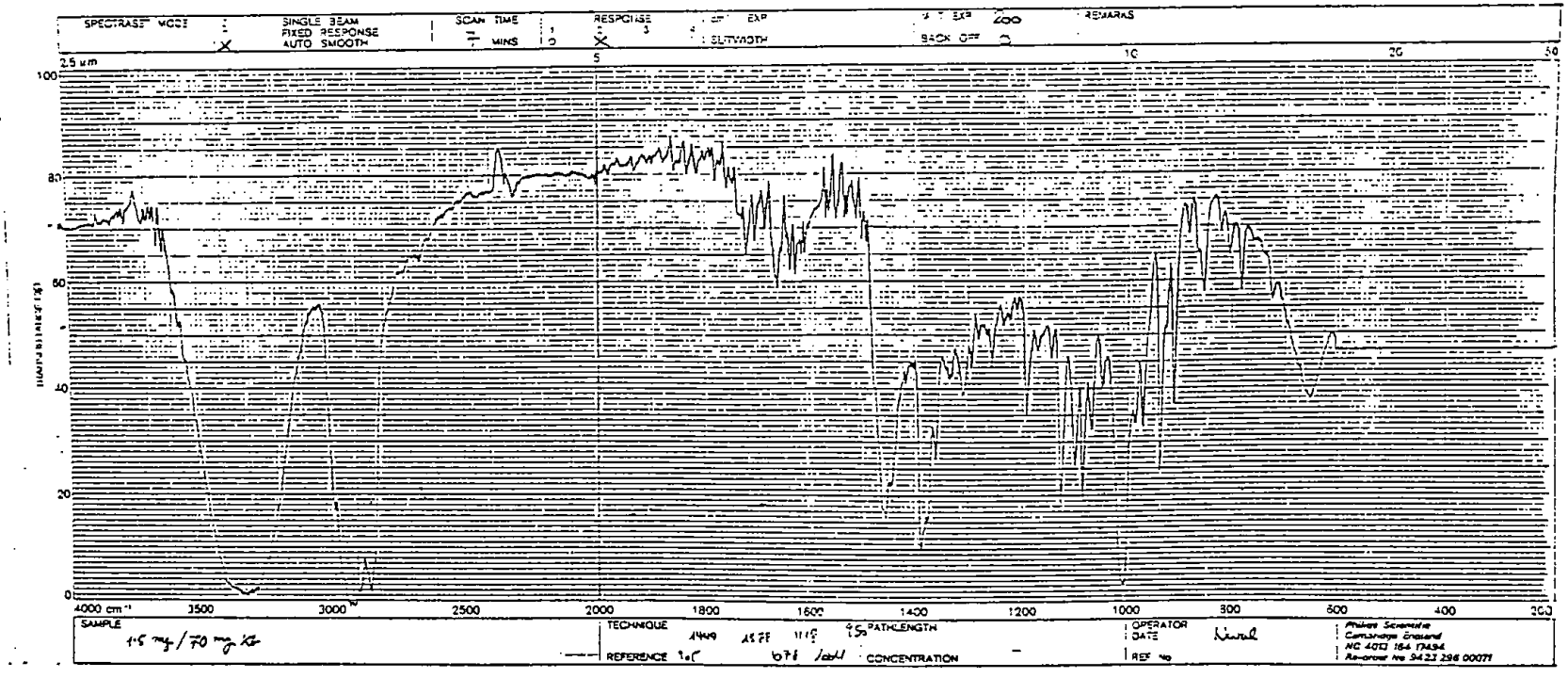


Fig. 23. Infra-red spectrum of the compound 2.

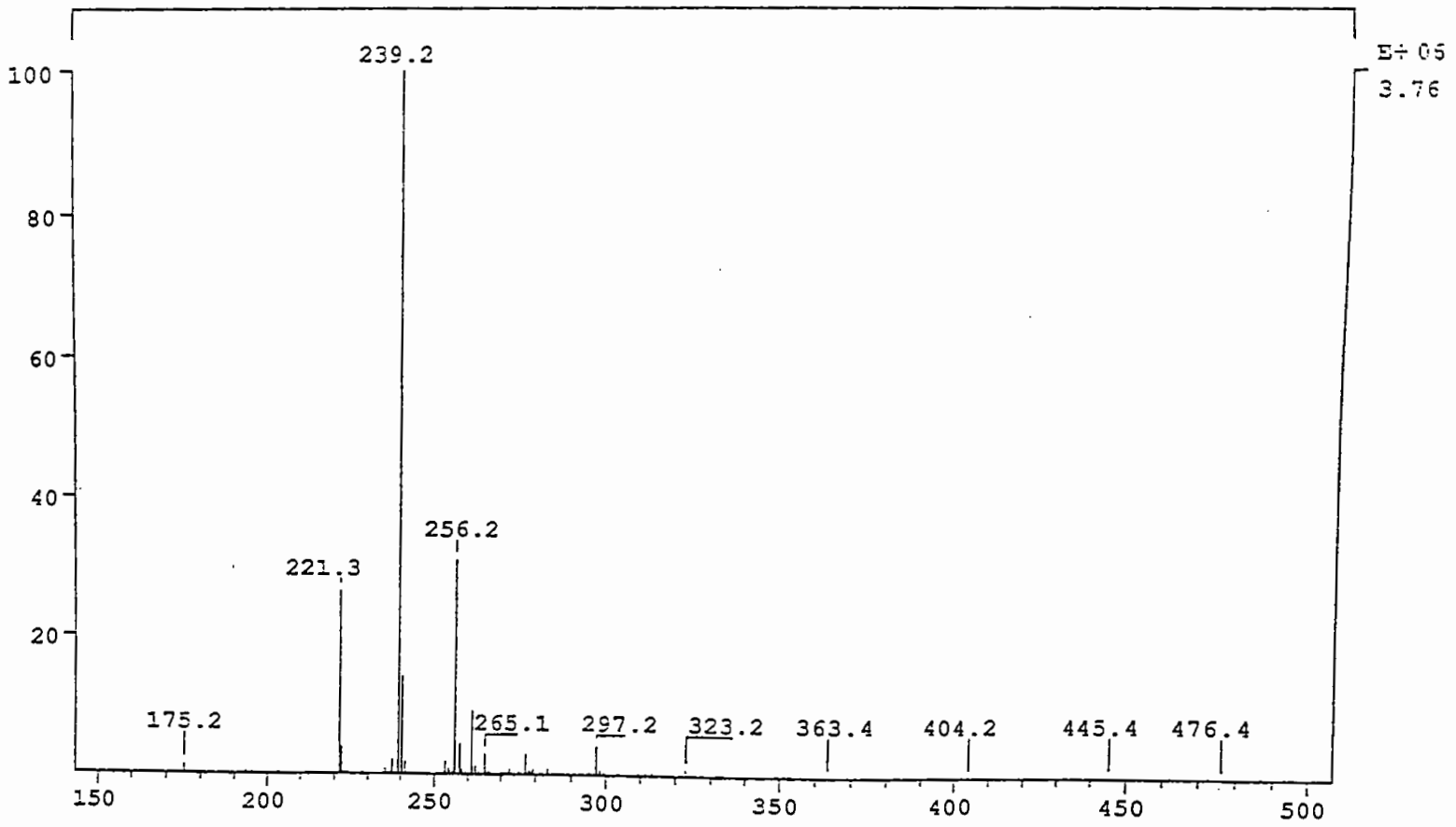


Fig. 24. Mass spectrum of the compound 1.

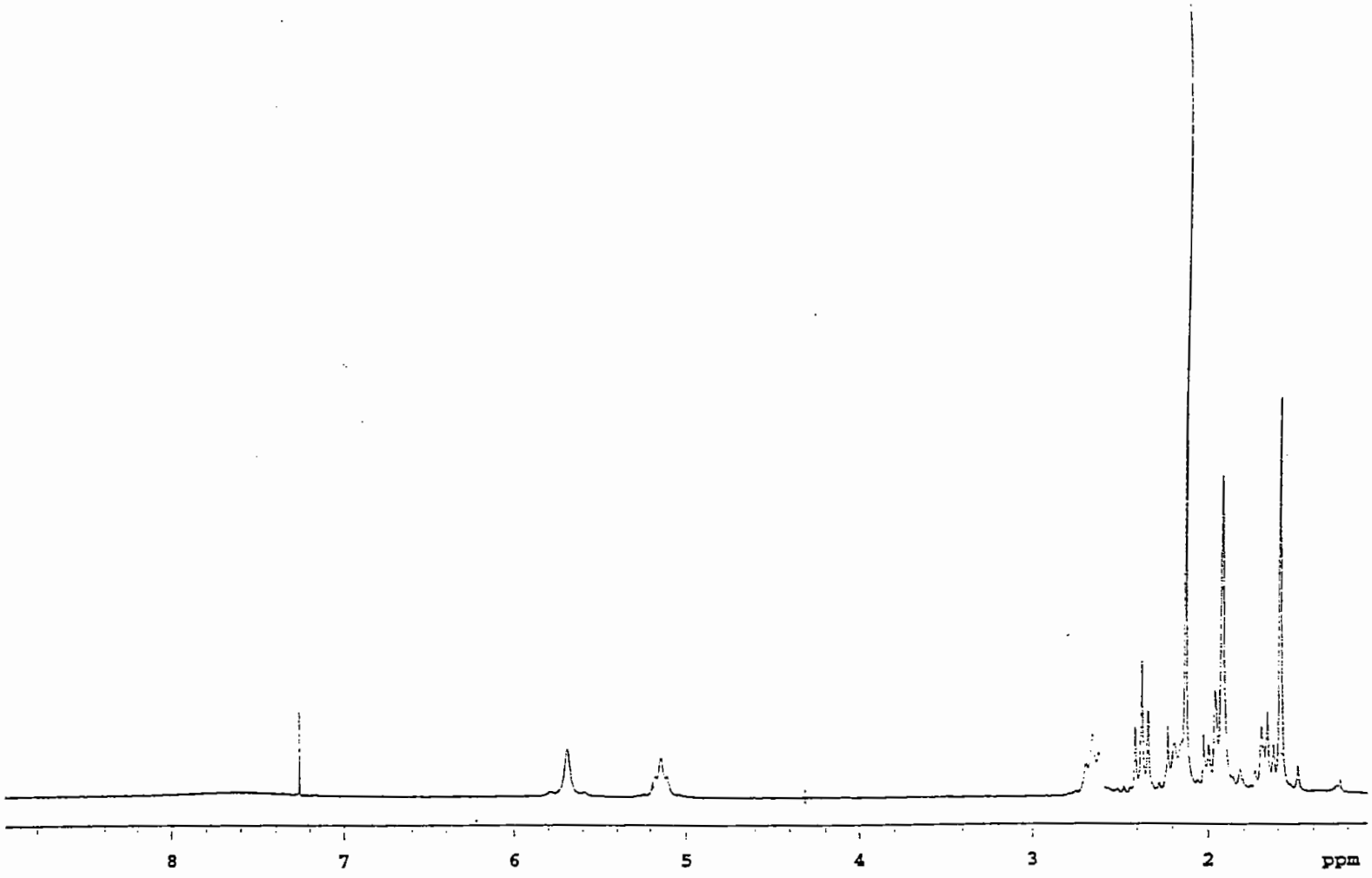


Fig. 25. ^{1}H-NMR spectrum of the compound 1.

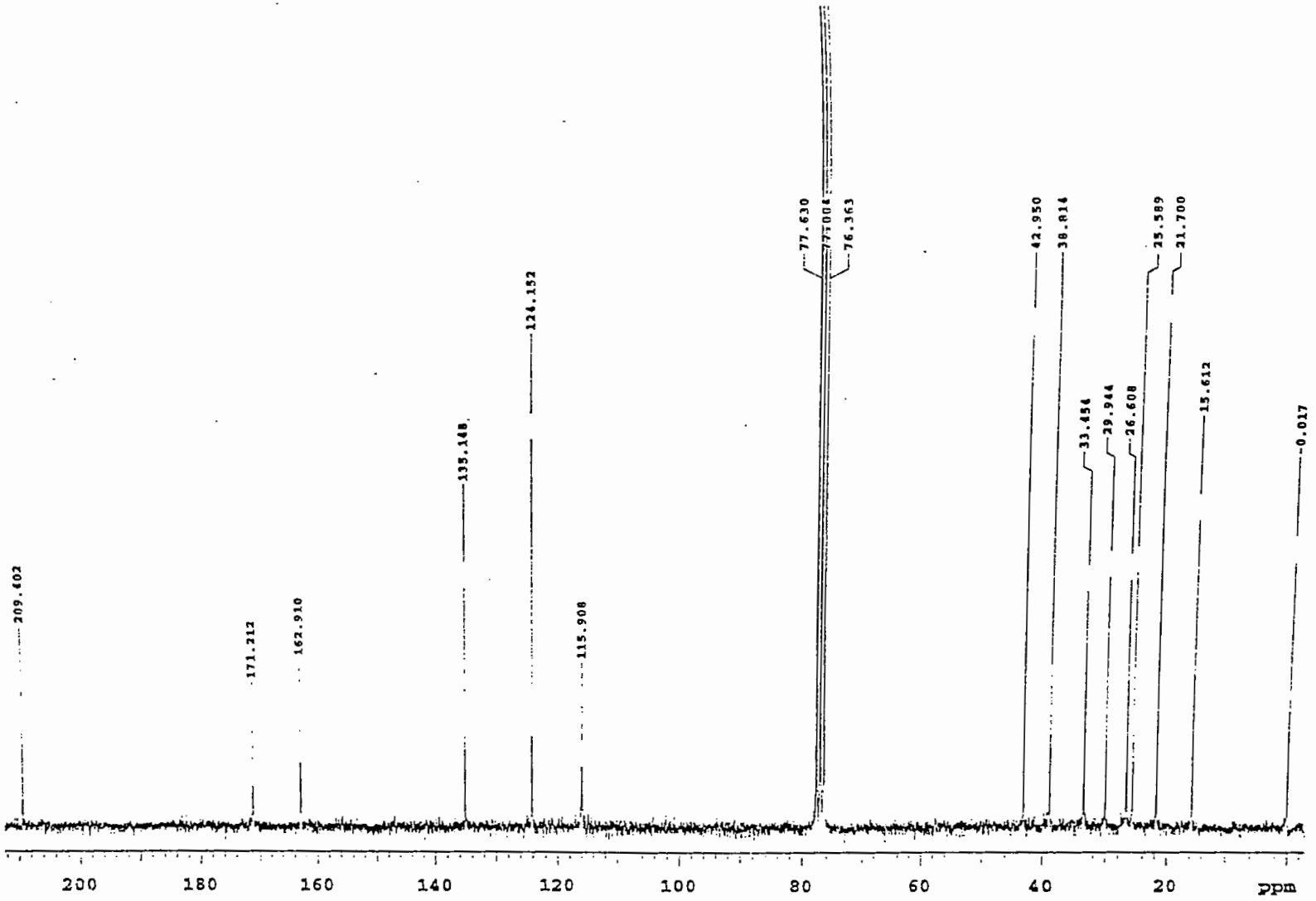


Fig. 26. ^{13}C-NMR spectrum of the compound 1.

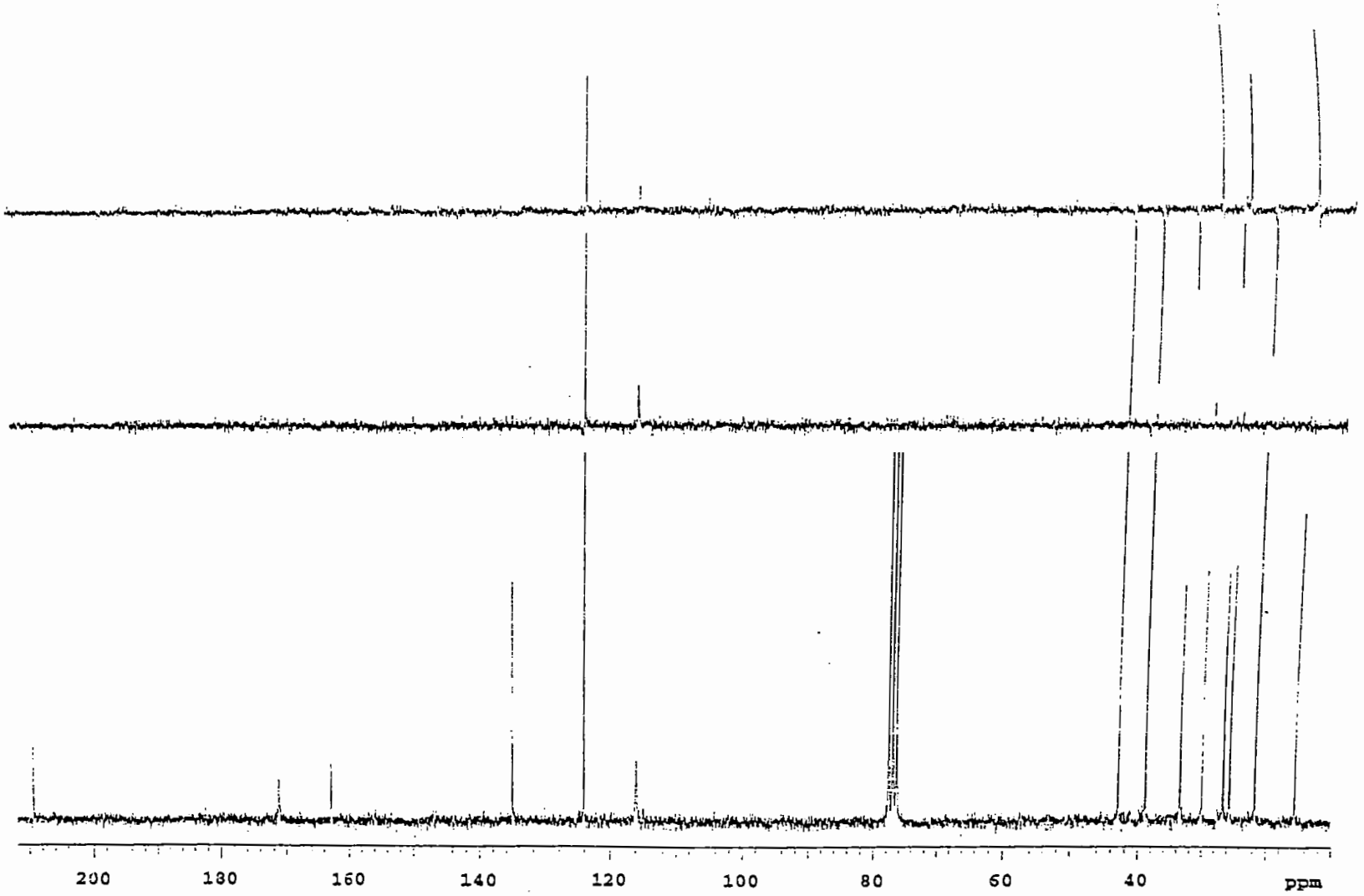


Fig. 27. ^{13}C-NMR spectrum for DEPT of the compound 1.

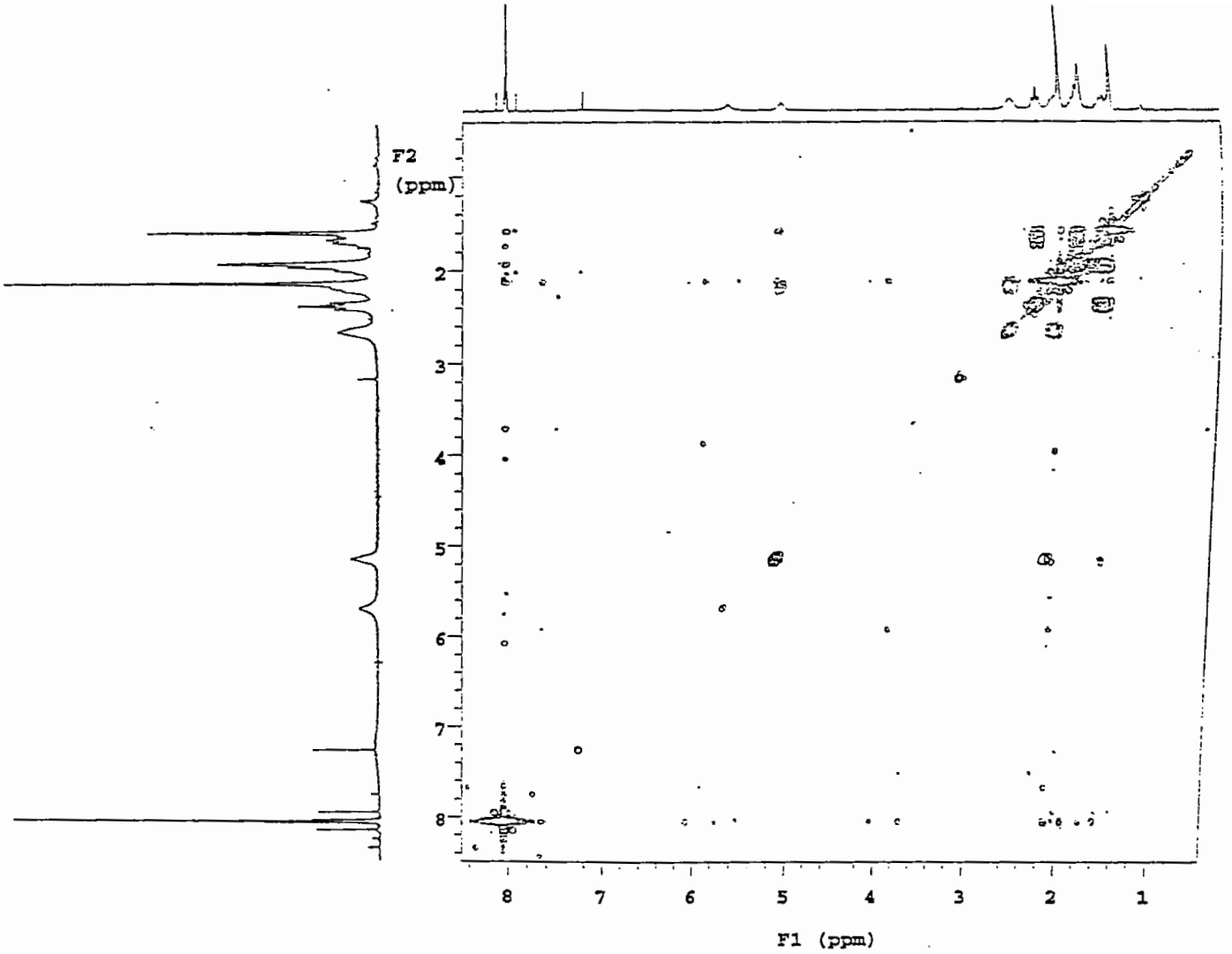


Fig. 28. ^{13}C-NMR spectrum for COSY of the compound 1.

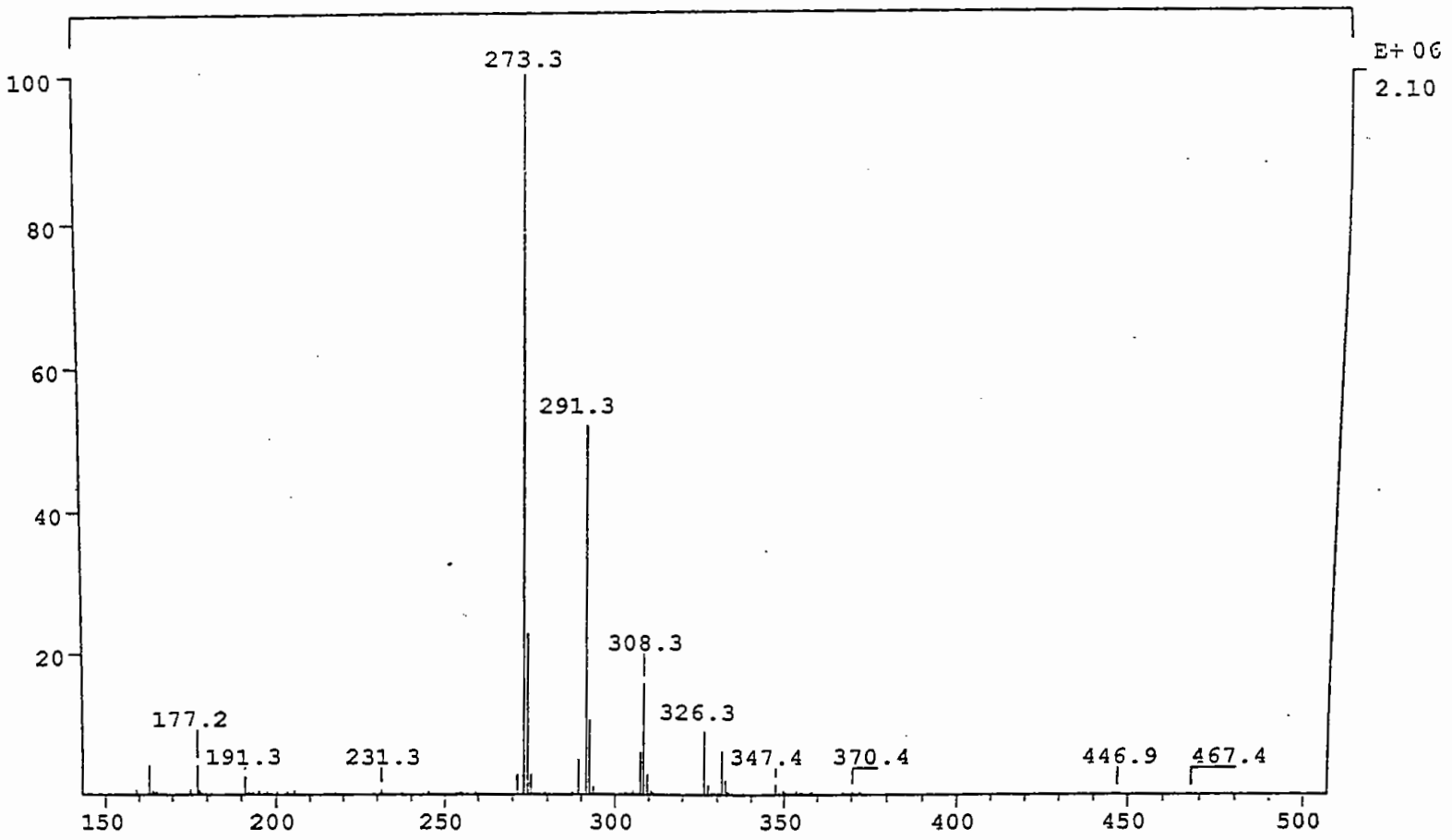


Fig. 29. Mass spectrum of the compound 2.

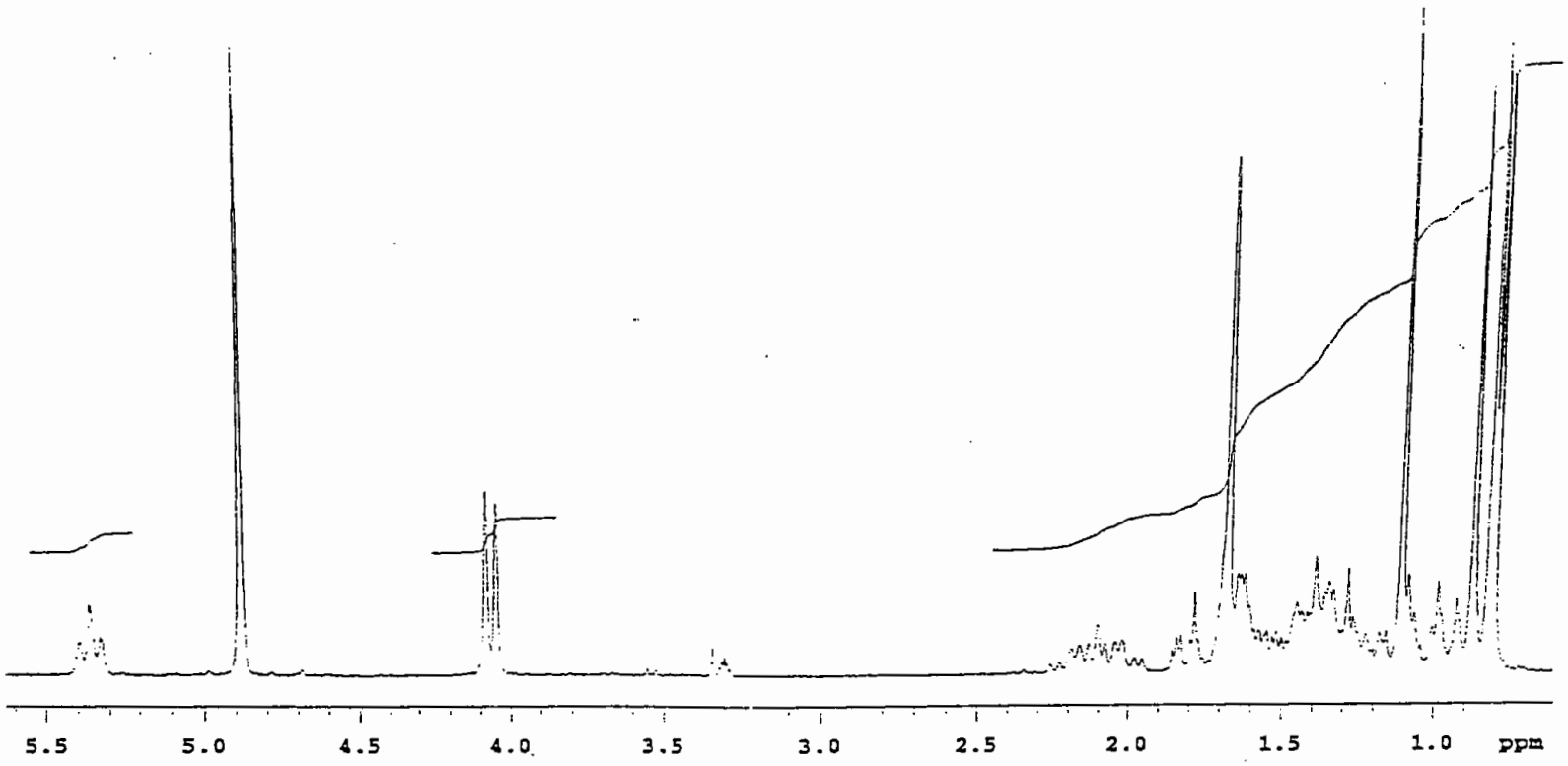


Fig. 30. ^{1}H-NMR spectrum of the compound 2.

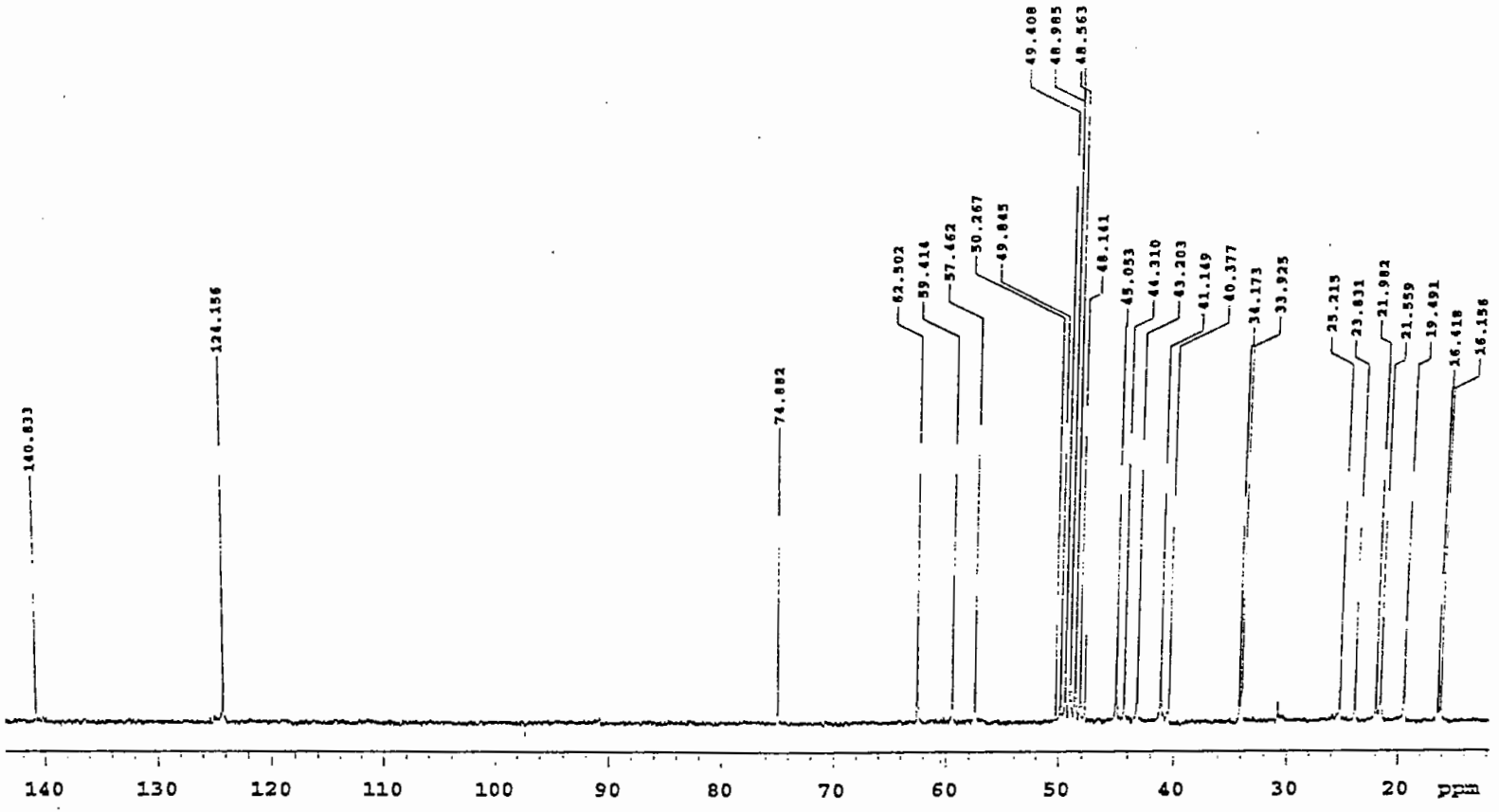


Fig. 31. ^{13}C-NMR spectrum of the compound 2.

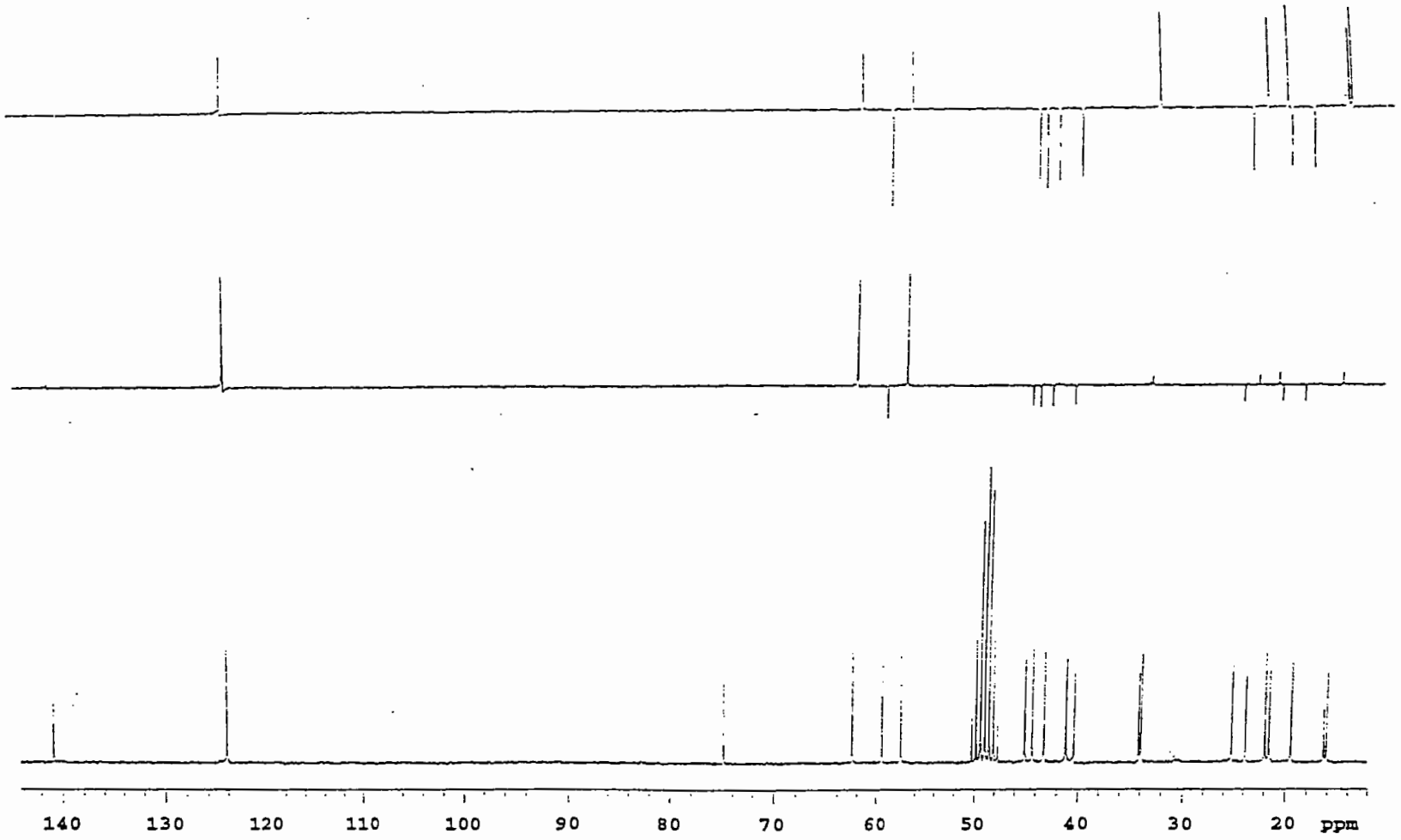


Fig. 32. ^{13}C-NMR spectrum for DEPT of the compound 2.

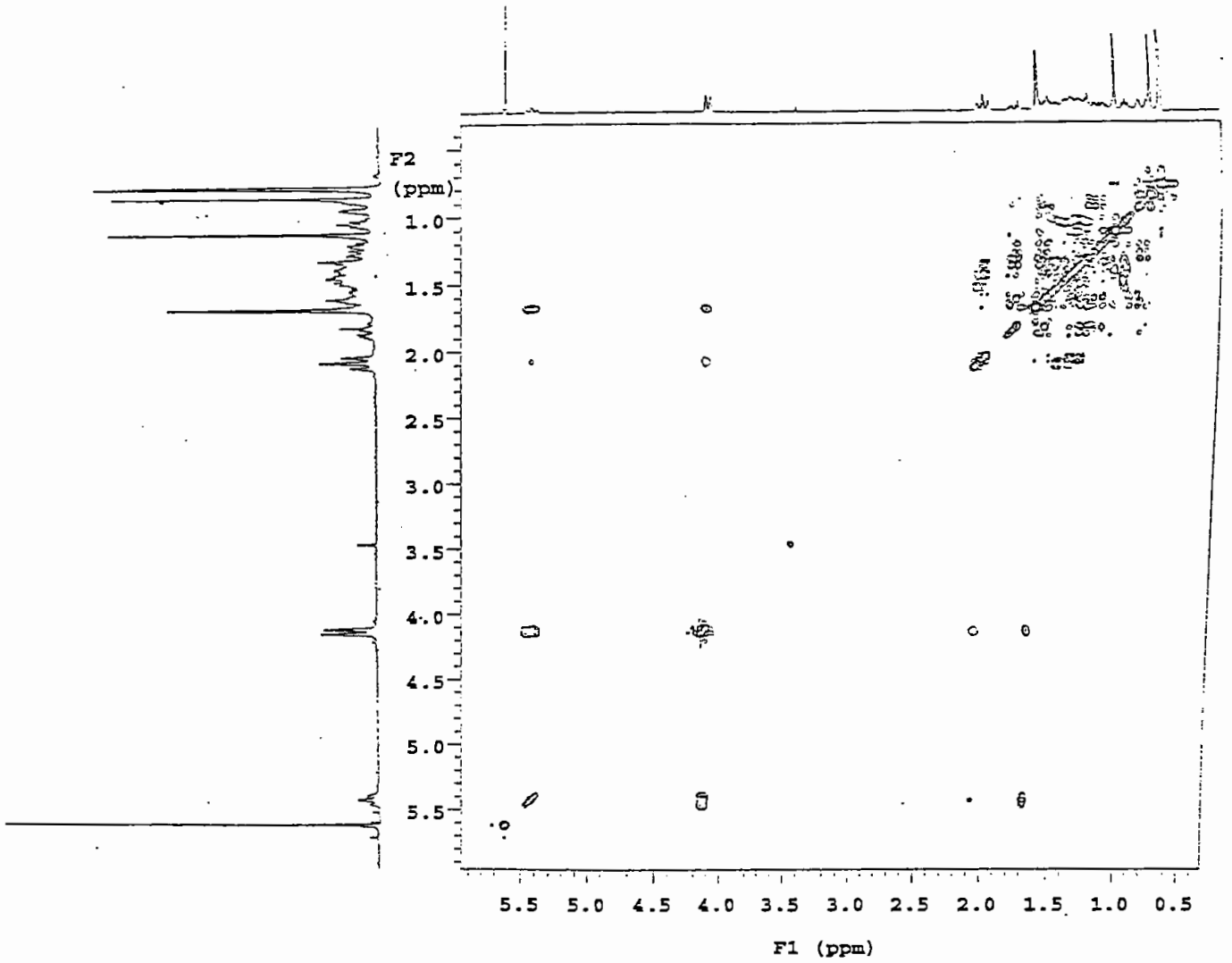


Fig. 33. ^{13}C-NMR spectrum for COSY of the compound 2.

3.5.4. Crystallization

For the compound 2 which was solidified soon after lyophilization followed by evaporation, crystallization was possible in a mixture of Hexane and iso-propanol; (1:1) with high concentration of the compound setting the material for slow evaporation by the air.

3.5.5 Measurement of melting point

Melting point (uncorrected) of the isolated powder like substance (compound 2) was determined with a mettler FP80 hot-stage apparatus which was 130°C and the same with the reported compound 'aphanamixol' firstly claimed by Chandrasekharan and Chakrabortty, (1968).

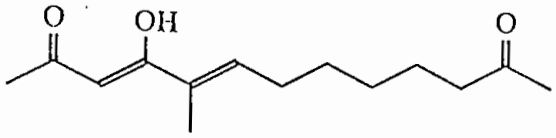
3.5.6. Determination of the structure of the isolated compounds

3.5.6.1. Structure of the compound 1

The structure of the compound 1 can tentatively be assigned to "4-Hydroxy-5-methyl undecadienedione" from its mass, ^{1}H and ^{13}C-NMR spectral analyses. From its mass spectrum a molecular ion peak appeared at M/z 239 (M^{+}). So, the molecular weight of the compound is 238 which corresponds with the molecular formula $C_{14}H_{22}O_3$.

In its ^{1}H-NMR spectrum one olefinic proton appeared at δ5.65 as broad singlet which can be assigned to olefinic proton of C-3 position which showed a slight long range coupling with the methyl groups of C-1 and C-5 position. Another olefinic proton appeared at δ5.15 as broad triplet assigned to be C-6 olefinic proton showed a coupling with the vicinal two protons of C-7 position and with the methyl group at C-5. Three methyl protons appeared as singlet at 1.60. 1.93 and 2.14. Other CH_2 peaks appeared in the high field between 1.40 to 280 ppm region.

There are 14 carbon signals present in its complete decoupled ^{13}C-NMR spectrum indicating the presence of 14 carbons in the compound. Of these, 3 quaternary carbon signals appeared in the low field region at 209.4, 171.2 and 162.9 ppm. which can be assigned as the two carbonyl carbon and a >C-OH carbon. From its DEPT spectrum another quaternary carbon of C-Me group appeared at 135.2 ppm. Two olefinic carbons appeared at 124.2 and 115.9 ppm. There are three methyl groups were present in the compound as indicated from its ^{1}H-NMR spectrum. ^{13}C-NMR DEPT spectrum also confirmed the presence of three methyl groups and their carbon signals appeared at 15.6, 25.6 and 29.9 ppm. Other 5 CH_2 carbons appeared at 21.7, 26.6, 33.5, 38.8 and at 43.0 ppm. From the above experimental data the compound 1 can tentatively assigned to be 4-hydroxy-5-methyl undecadienedione.

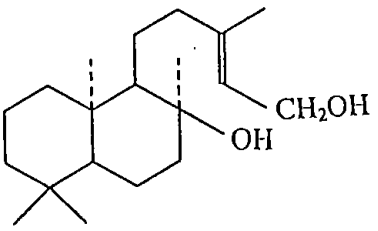


4-hydroxy-5-methyl undecadienedione

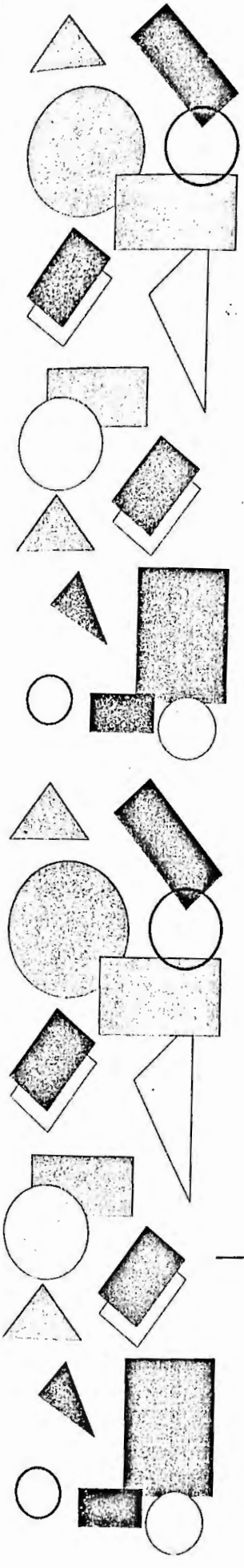
3.5.6.2 Structure of the compound 2

From its mass spectrum the compound 2 has a molecular ion peak appeared at M/z 308 (M^+). So, the molecular weight of the compound is 308 which corresponds with the molecular formula $C_{20}H_{36}O_2$ ($[M]^+$ M/z 308). Its IR spectrum showed the presence of hydroxyl group (3310 cm^{-1}) and tertiary 6-methyl (1449 cm^{-1}) and gem-dimethyl group (1378 cm^{-1}). Other bands are 1118, 1078, 1004, 930 and 905. The ^{1}H-NMR spectrum of compound 2 exhibited five singlets at δ0.83, δ0.84, δ0.88, δ1.12 and δ1.68 corresponding to 5 methyls, a triplet at δ5.36 due to an olefinic proton and a doublet at δ4.07.

There are 20 carbon signals present in its complete decoupled ^{13}C-NMR spectrum indicating the presence of 20 carbons in the compound. Of these two quaternary carbons present at 41.2 and 34.2 ppm, one carbonyl carbon signal appeared at 140.8 ppm. one >C-OH carbon at 74.9 ppm, three olefinic carbon signals appeared at 124.2, 62.5 and 57.5 ppm. There are five methyl groups were present in the compound as indicated from its ^{1}H-NMR spectrum. 13C-NMR DEPT spectrum also confirm the presence of five methyl groups and their carbon signals appeared at 33.9, 23.8, 22.0, 16.4 and 16.2 ppm. Other 8 CH_2 carbons appeared at 59.4, 45.1, 44.3, 43.2, 40.3, 25.2, 21.6 and 19.5 ppm. From the above mentioned data the compound 2 can be assigned as a diterpene alcohol which was isolated and named as 'aphanamixol' by Chandrasekharan and Chakrabortty (1968). Jefferies and Payne (1965) showed a compound with the same molecular formula as 13-Epi.(-)- manoyl oxide isolated from the family *Euphorbiaceae*. The same compound also reported by Rojatkar *et al.*, (1994), from the aerial part of *Cipadessa fruticosa* designated as a labdane derivative and from *Grangea maderaspatana*, a herb belonging to the family *Asteraceae*.



Aphanamixol



Chapter 4

Discussion

Chapter 4 Discussion

All the extractives from leaves, seeds, fruit-pericarp, stem-bark and roots of the title species *A. rohituka* collected in Pet. spt., EtOAc, acetone and MeOH were tested on the red flour beetle *T. castaneum* according to the title proposal, and all were found more or less effective on almost all the strains of the test insect. The activity found on all the three strains viz. CR-1, FSS-II and CTC-12 of *T. castaneum* adults and pupae not satisfactory since there was nothing to comment or predict about the plant extractives. There was no distinct scope of showing differences of action of the doses on the different strains of the test insect, while some distinguished differences were found in case of pupal mortality and adult mortality, as well as, recoveries were recorded in particulars steps.

Talukder and Howse (1994, 1995) studied the repellent, antifungal, toxicant and protectant characteristics of *A. polystachya,* (Syn.= *A. rohituka*) on *T. castaneum* and observed that seed extracts showed strong repellent effects. Among the four extracts they tested, acetone extract showed highest repellent effect on beetle 100%, followed by MeOH extract 98%, EtOH extract 95% and Pet. ether extract 92% respectively. They (1995) also studied the contact toxicity to red flour beetle for four extracts as EtOH> acetone>MeOH>Pet. ether extracts. But in the present investigation highest mortality was

recorded in case of the Pet. spt. extract of the roots, where LD_{50} values were ranging between 0.657 µg/µl (on CR-1 pupae for 24 h) to 2.156 µg/µl (on FSS-II pupae for 72 h) and 1.897 µg/µl (on CR-1 adult for 24 h) to 7.271 µg/µl (on FSS-II adult for 48 h) (Table 22).

Acetone and MeOH extracts offered less mortality among all the experiments, and acetone extracts were found comparatively more active than MeOH extracts. Lowest mortality was recorded in case of MeOH extract of roots.

In contact toxicity test Talukder and Howse (1995) observed that EtOH extract of Pithraj seeds was the most toxic to red flour beetle followed by acetone extract. Methanol was the least toxic followed by Pet. ether extract. This proved matter was reported earlier by Islam, (1983) by which he established the scientific conception about utilities of the title plant which are still prevalent in folk use by our people for centuries together. Khan and Hassan (1981) wrote a note book in Bangla for the army personnel to let them be aquatinted with the toxic plants of Bangladesh, in which he didn't mention the name of *A. rohituka*. So, it is a fine attribute to have properties from a non-toxic but biologically active plant. In the present investigation the seed extracts showed contact toxicity in the order of EtOAc> Pet. spt.> acetone> MeOH in general, while all tests have been carried out on three standard strains of *T. castaneum* pupae and adults for 24-, 48- and 72- hours of treatment.

The contact toxicity of pithraj fruit-pericarp extracts of Pet. spt. was the highest to red flour beetles. Mortality was directly proportional to the level of concentration and to time. Among the tested extracts, Pet. spt. extract showed the highest toxic effects and lowest LD_{50} values in case of all the strains. Higher concentrations contributed more significantly to the efficacy of extracts on the mortality of insects and concentration appeared to the degree of control obtained with pithraj friut-pericarp extracts. Khanam *et*

al., (1990) has reported deleterious effect of royna seed-coat on the growth and development of *T. castaneum* and observed reduced larval, pupal and adult weight. It is necessary to mention that pithraj and royna are the vernacular synonyme of the meliaceous species *A. rohituka.*

To highlight the significance of the activities found among the extracts of different parts of the title plant it is necessary to discuss about the components have been reported from different organs of this plant by indicating the previous media and procedures of their collection. With the information on the probable component and constituents reported it could be found that some active compounds offered referable activities on *T. castaneum.* The compounds found in the title plant are described here indicating their activities if stated by the previous authors.

Petroleum spirit extract of the leaves upon chromatographic resolution over neutral alumina using chloroform as the eluent yielded aphanamixol (structure a, Chart 2). It has the molecular formula $C_{20}H_{36}O_2$ (M^+308), m.p. 130°, and $(\alpha)_D$-11°. The compound showed the absence of phenolic, methoxyl and carbonyl groups from spectral and chemical data. The infrared spectrum provides several important information regarding the functionalities of this aphanamixol. The presence of hydroxyl unsaturation, tertiary C-methyl and gem-dimethyl groups is revealed from the appearance of bands at 3300, 1666, 1408 and 1380 cm^{-1} in the IR spectrum (Chandrasekharan and Chakrabortty, 1968).

It was a crystalline neutral compound. The isolation of the same substance was reported by Jefferies and Payne (1965) from the leaves of *Beyeria* species, of the family *Euphorbiaceae* and the authors designated this compound as eperu-13-en-8β:15 diol; a diterpenoid of manoyl-oxide series and it was found as compound 2 in this investigation and found active against *C. albicans* and *B. subtilis.*

Ethanol extraction of dried leaves and stems, followed by acid base work up, furnished dark-brown resinous material and fractionation of this extract by column chromatography (silica gel; methylene chloride/MeOH, 2:1) afforded three widely separated basic fractions. Rohitukine (structure b, Chart-2) was isolated from the most polar band and was obtained in crystalline from upon slow evaporation of the eluting solvent. Recrystallization from MeOH gave pale yellow crystals, m.p. 218-219°C. High resolution mass spectrometry and elemental analysis established the molecular formula $C_{16}H_{19}NO_5$ (M^+305, 1264; calculated 305, 1264). Mass fragmentation indicated the loss of CO and H as a major pathway. The IR spectrum of rohitukine established the presence of hydroxyl and ketone groups and suggested the presence of a Y-pyrone. The nitrogen is tertiary (absence of N-H stretching bands). It is the first example of a chromone alkaloid (the flavone alkaloids ficine and iso-ficine) from *Ficus pantoniana*, (*Moraceae*), and it seems to be the first alkaloid from the family *Meliaceae* (Harmon *et al.*, 1979). They (1979) made an important comment on this compound that its isolation from the *A. rohituka* extract does not prove that it occurs as such in the plant, rather it could have formed from one or related alkaloids during the prolonged storage of the extract. A literature search at this stage revealed that an antiinflammatory cum immunomodulatory piperidinylbenzopyranone was isolated from *Dysoxylum binectariferum* which was constitutionally was the same as the alkaloid rohitukine (Naik *et al.*, 1988).

A limonin compound has been isolated from the seed of *Aphanamixis polystachya*. m. p. 275-280°C (decomp.) $[\alpha]_D$-31°, $C_{34}H_{42}O_{13}$ as indicated by Connolly *et al.*, (1976), from the family *Meliaceae*, which they named rohitukin (structure c, Chart 2). For this limonoid they proposed a structure, whose ^{1}H-NMR spectrum is very similar to that of prieurianin.

The seeds extracted by refluxing with EtOH (95%) for 15 days, condensed under reduced pressure and poured into distilled H_2O with continuous stirring. The water soluble portion on extraction with C_6H_6 gave a saponin. It was purified on a column of neutral alumina, crystallized as white needles with $CHCL_3$-MeOH, m.p. 65-66°. The homogeneity of the saponin was checked by PC in nBuOH-HOAc-H_2O (4:1:5), Rf 0.40. IR Vmax (Cm^{-1} : 3420 (OH group), 2952, 1050, 1575 and 800 (strong) (Δ^{24}(28) ethylidene sterol), 1475, 1390 and 995 (iso-propyl group). ^{1}H-NMR ($CDCl_3$) : δ0.81 (S, H -19), 0.99 (fused d, H-21, H-26 and H-27), 102 (S, H-18), 1.55 (d, H-29), 2.81 (septet, H-25), 5.02 (q, H-28) and 4.7 (br.m. H-6). The isolated new saponin was stigmasta -5, 24(28)-dien-3β-0-β-D-glucopyranosyl -0-α-L-rhamnopyranoside, (Bhatt *et al.*, 1981).

Seeds produced by the Haimalayan *Meliaceae* species *Aphanamixis grandifolia* Br. have been shown to contain two new and highly cytotoxic limonoids assigned structures as aphanastatin (structure k, Chart-3) and amoorastatin (structure j, Chart-3) and both substances were found strong to inhibit growth of Murine P-388 lymphocytic leukemia (Polonsky *et al.*, 1979).

Separation directed by bioassay of the *A. grandifolia* seed extract (aqueous) led to discovery of a new highly cytotoxic limonoid aphanastatin against Murine P-388:ED_{50} 0.065 *μl*/ml. A chloroform-soluble fraction of the water extract was subjected to successive gradient elution (Chloroform:Methanol) chromatographic separations on silica gel (E. Merck) to afford this product as crystals (from Chloroform-Methanol) decomposing at 269-271°C, $[\alpha]^{22}_D$-389° (C. 0.46, 1:24 Pyridine:Methanol), and CDΔ^e-2.88 (311 nm) in the same solvent, corresponding to molecular formula $C_{35}H_{46}0_{13}$ (mass spectrum M/z 674.2907 for m^+) and this may be an α-methyl butyrate diacetate derivative of a tetranortriterpene. The ^{1}H-NMR data also indicated the presence of a furan ring and

double resonance experiments suggested presence of the system -CH(OAc)-CH(OH)-CH(OAc)-, (Polonsky *et al.*, 1978a).

Further investigation of the same extract (directed by bioassay) has led to isolate a new limonoid designated as amoorastatin (structure b, Chart 3), with even greater cell growth (P388) inhibitory (ED_{50} = <0.001μg/ml) properties, m.p. 205°C (sintering from 170°C, crystals from $CHCl_3$-MeOH), CD in dioxane, Δ^e-4.01 (300 nm) and -3.64 (310 nm). High resolution mass spectrometry allowed assignment of molecular formula $C_{28}H_{36}O_9$ (M^+ M/z 516.2362). Amoorastatin crystallized with 1 mol of water (m-p 170°C) in the monoclinic form with space group P_{21}; a = 7.780 (2), b = 12.818 (2), C = 13.003 (3) Å, β = 91.51(3)°, (Polonsky *et al.*, 1978b).

Much interest has been focused on toxic principles of various meliaceous plants which have traditionally been employed as fish or dart arrow poisons. The major toxic component of *Aphanamixis polystachya* was a known triterpenoid, aphanamixin (structure e, chart 2). The petrol-extract of the bitter fruit-shell furnished this compound which has been isolated and purified by chromatography over Brockman alumina using petrol ether and benzene: chloroform mixture (1:1) as the eluents. Aphanamixin migrated out of the column with the latter solvent mixture and gave stellate crystals from methanol. The elemental analysis and the mass spectrometrically derived molecular weight (M^+ 514) of the compound established its molecular formula as $C_{32}H_{50}O_5$, m.p. 232-234°, $[\alpha]_D$ -45° ($CHCl_3$). It contains an OAc group (1720 and 1260 cm^{-1}) and a OH (3380 cm^{-1}) which forms an acetate (21, 23R, 24R, 25-diepoxytirucall-7-ene-3β-21β diol diacetate), $C_{34}H_{54}O_6$, m.p. 128-130°. The NMR spectrum of aphanamixin indicates the presence of seven tertiary Me groups [singlets at δ1.31 (6H), 0.78-1.00 (15H)]. One OAc [singlet at

δ2.06 (3H)] and a OH function [singlet at δ3.24 (1H)] which disappears when refluxed with D_2O (Chatterjee and Kundu, 1967; Chatterjee *et al.*, 1970).

The same compound as a major toxic component and two other minor toxic principles, aphanamol I and aphanamol II (structure n,o, Chart 3) were isolated from *A. grandifolia* fruit-shell. Dried peel of *A. grandifolia* was crushed and extracted with hexane in a soxhlet apparatus for 48h to give a crude extract. In this case fractionation was always monitored by toxicity against fish, *Oryzias latipes*. The major toxic constituent aphanamixin, showing a toxicity of LC_{50} 0.06 ppm (48h) (Pettit *et al.*, 1983), was easily isolated by silica gel column chromatography followed by recrystallization in 1.1% yield.

The minor toxic components, aphanamols I and II, were isolated after consecutive purification by normal phase (silica gel, hexane-EtOAc) and reverse phase (Fuji-Devison Micro Beads Silica gel 5D-ODS, 100-200 mesh, acetonitrile-water) column chromatography and finally by HPLC (Nomura Chemicals Develosil ODS-5 column, Altex RI detector, acetonitrile-water).

Aphanamol I obtained as colorless oil [0.015% yield, LC_{50} 28 ppm (48h); $C_{15}H_{24}O_2$; $[\alpha]^{18}_D$ +13.8° (C 0.29, $CHCl_3$)] showed hydroxyl (3400 cm^{-1}) and carbonyl (1690 cm^{-1}) absorption in the IR spectrum.

Aphanamol II was obtained as colorless oil in 0.005% yield [LC_{50} 27 ppm (48h); $[\alpha]^{18}_D$ + 6.9° (c. 0.94 $CHCl_3$); IR (neat) 3450, 2720, 1680 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ0.92(3H, d, J = 7 Hz), 0.93 (3H. d. J = 7 Hz), 1.04 (3H, s) 3.40 (1H, dd, J = 6Hz), 6.62 (1H, d, J = 5Hz) 9.37 (1H, s). The structure of the aphanamols represents a very rare type of carbon skeleton for naturally occurring sesquiterpenoids.

A new triterpenoid, aphanamixinin (structure g, Chart 2), $C_{27}H_{34}O_7$ (M^+ 470). m.p. 208°, $[\alpha]_D$- 120° ($CHCl_3$), isolated from the petrol extract of the dried bark shows bands in its IR spectrum at 1510 and 875 cm^{-1} (firm ring). a broad band in the region 1715-1740 cm^{-1} (carbonyl, ester and/or lactone functions), but no peak absorptions for OH. The NMR spectrum shows signals at δ 6.35 (1H) and 7.38 (2H) corresponding to one β-furanoproton and two α-furnoprotons respectively, a singlet at δ 3.75 (3H) for a methoxycarbonyl group, four protons associated with singlets (δ 5.65, 5.10, 4.90 and 3.82) and four tertiary C-Me groups (δ 1.15, 1.10, 1.05 and 0.90).

Aphanamixinin is a lactone and consumes one equivalent of alkali in the cold and is regenerated from the alkaline solution upon acidification. Alkaline hydrolysis of aphanamixinin with alcoholic alkali produces aphanamixinic acid, $C_{26}H_{32}O_7$ which with diazomethane regenerates the parent compound, thus proving aphanamixinin as the methyl esters of aphanamixinic acid. (Chatterjee *et al.*, 1970).

Aphanamixinin, was also reported from the petrol extract of the dried bark of *A. palystachya.* Air dried and powdered stem-bark of *A. polystachya* extracted exhaustively thrice to rectified spirit under reflux of 30 days and was concentrated under reduced pressure and segregated into water soluble and insoluble fractions. The water insoluble material was extracted with Pet. ether (b.p. 60-80°) gave a mixture of 3 compounds (on TLC) which were separated on Al_2O_3 column to yield compound the 1st (Hexane:Pet. ether; 9:1) a *β*-sitosterol, the 2nd (Hexane:Pet. ether; 7:3) a stigmasterol and the 3rd (Pet. ether) characterized as $C_{41}H_{68}O_{10}$. m.p. 138-140, $[\alpha]_D^{25}$ +53° ($CHCl_3$), (M^+ 442) gave all positive tests for a saponin, which the authors later called aglaiol-3-O-rhamnosyl-xyloside. (Srivastava and Agnihotri, 1984 and 1985).

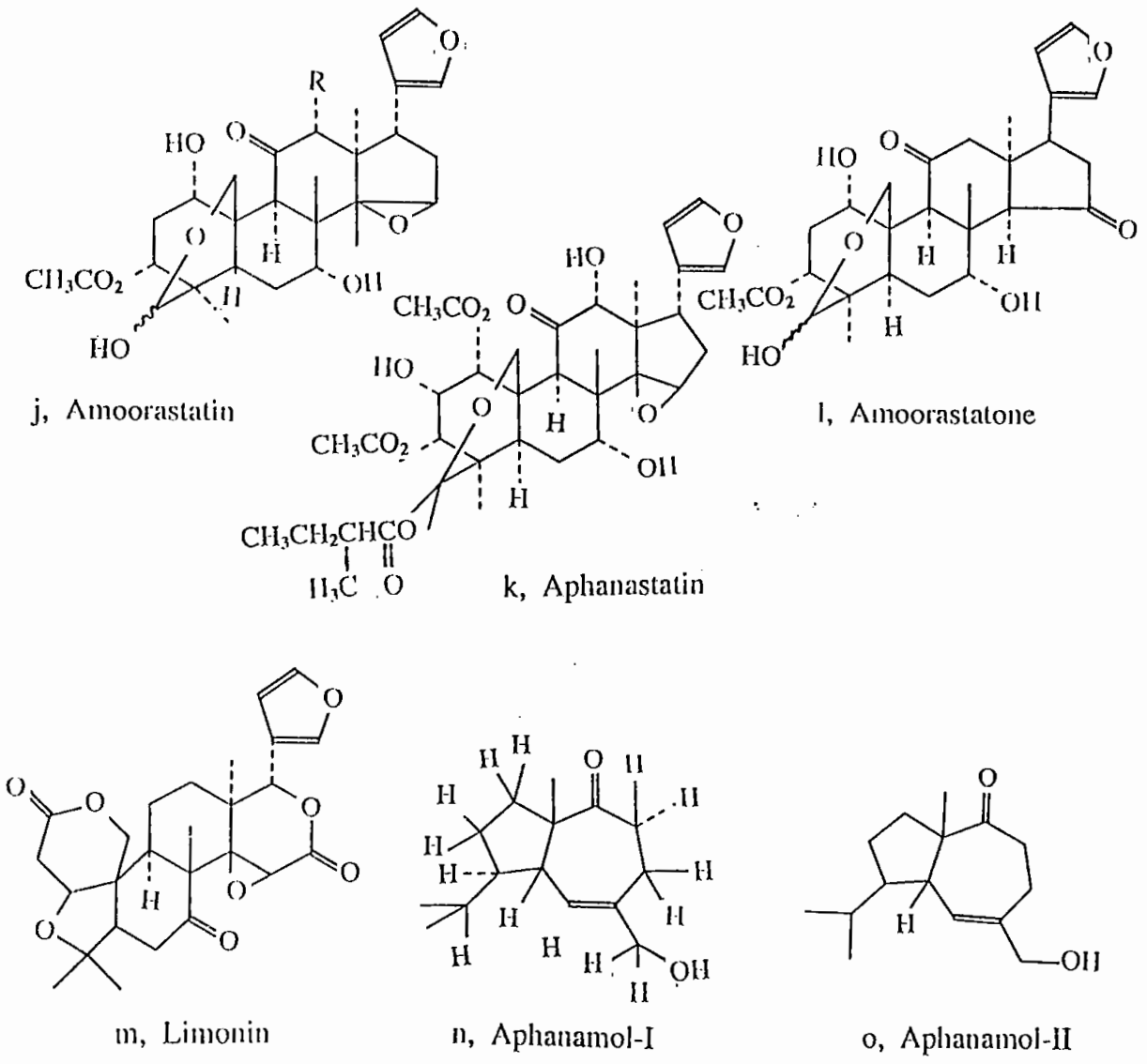


Chart 3. Bio-active constituents reported from other related species of the title plant.

The continuous study led by the same authors offered two new glycosides. The water insoluble fraction was successively extracted with Pet. ether, C_6H_6, $CHCl_3$, EtOAc and Me_2CO. The EtOAc and Me_2CO extracts yielded the 4th and 5th compounds D and E respectively. Both the compounds were purified over silica gel column (MeOH:Me_2CO; 5:5) and crystallized (Me_2CO:Et_2O).

The 4th compound was a reddish brown amorphous solid, $C_{23}H_{24}O_{12}$, m.p. 158-162° gave positive tests for an anthraquinone glycoside. Rf 0.68 ($CHCL_3$:MeOH; 7:3) and Rf 0.85 (Me_2CO:MeOH; 1:9) on TLC. The 5th compound was assigned as 1.5-dihydroxy-6,7,8-trimethoxy-2-methyl-anthraquinone-3-O-β-D- xylopyranoside.

Another compound, the 6th, was an yellow brown amorphous substance, $C_{23}H_{26}O_9$, m.p. 125-128° (d), gave all the positive tests for a flavanone ghycoside. On LTC. Rf. 0.62 ($CHCl_3$: MeOH; 7:3), 0.73 (Me_2CO:MeOH; 1:9). This compound is assigned as naringenin 7,4-dimethyl ether-5-0-α-L-rhamnopyranoside (Srivastava and Agnihotri, 1985).

A new saponin reported by Agnihotri (1987) has been isolated from air dried and powdered stem-bark of *A. rohituka* exhaustively extracted thrice with ethanol under reflux for 180h in EtOH. After reducing the amount the extract was then segregated into water soluble and water insoluble fractions. The water soluble fraction was extracted with Pet. ether and benzene respectively. The benzene extract was purified over Al_2O_3 column (C_6H_6:$CHCl_3$; 5:5) and crystallized (MeOH:ether) as a reddish-brown crystalline substance, m.p. 122-25 (dec.), $C_{35}H_{58}O_5$, $[\alpha]_D^{30}$ + 16.3° ($CHCl_3$) which gave all positive tests for a saponin. The compound was assigned as a poriferasterol-3-rhamnoside.

The powdered stem-bark of *A. rohituka* was exhaustively extracted with ethanol under reflux on a water bath for 200h. The ethanol from the percolates was removed under reduced pressure and kept at room temperature for a few days during which time a dirty white mass was deposited. This was separated by filtration and passed through a column of neutral Al_2O_3. Elution with C_6H_6:$CHCl_3$ (1:1) and crystallization from $CHCl_3$ afforded colorless needles which was characterized as C; 68.87, H; 7.18; $C_{27}H_{34}O_7$ required C; 68.93, H; 7.23%); $[\alpha]_D$: -105°; ^{1}H-NMR O95, 100, 108, 1.10 (each, s, 12H,

$4CH_3$), 1.20-1.90 (complex pattern, CH_2 and CH), 3.20 (s, OH), 3.65 (d, j= 6Hz, 1H, H-3), 3.75 (s, 3H, $COOCH_3$), 3.80 (s, 1H, H-15). 4.90 and 5.10 (each s, 2H, $>C=CH_2$). 5.65 (s, 1H, H-17), 5.90 (dd, J=6 and 10 Hz, 1H, H-2), 6.15 (d, J = 10 Hz, 1H, H-1) 6.40, 7.20 and 7.39 (each m, 3H, Furan H); MS : 470 (M^+), 457, 452, 437, 411, 403, 402, 389, 263 and 245.

The compound $C_{27}H_{34}O_7$ (M^+ 470), m.p. 172-174°C $[\alpha]_D^{25}$: -105° (C 0.14, $CHCl_3$) exhibited in its IR spectrum absorptions characteristic for hydroxyl (3450) δ lactone (1735), ester (1725), trisubstituted double bond (1640, 825), furan (1510, 875), epoxide (1285), and exocyclic methylene groups (910 cm^{-1}). The ^{1}H-NMR spectrum suggests that this compound is closely related to aphanamixinin. This assigned as amoorinin (3-di-hydroandirobin) (structure f, Chart 2) on the basis of spectral and chemical methods.

A new flavone glycoside has been reported from the roots of *A. rohituka* by Jain and Srivastava, (1985). The air dried and powdered roots was extracted with ethanol under reflux for 120 h and then concentrated under reduced pressure and poured into H_2O with continuous stirring. The H_2O soluble fraction was concentrated to a syrupy mass that was than extracted with MeOH which yielded the reported glycoside, m.p. 360-365° (dec.). The compound was then purified over a column of magnesol (hydrated magnesium silicate) developed with MeOH and crystallized (MeOH-$CHCl_3$) as a dark brown amorphous solid (found C, 56.22; H, 4.40: $C_{21}H_{20}O_{11}$ required C, 56.25: H, 4.46); TLC silica gel, Rf 0.45(Me_2CO-MeOH, 8:2) and 0.28 (MeOH-$CHCl_3$; 5:5).

Molecular weight determination by mass spectral and elemental analysis established the molecular formula as $C_{21}H_{20}O_{11}$ (M^+ 448) and it gave all the positive

colour tests for a flavone glycoside. This compound is assigned as 8-C-methyl-quercetin-3-O-β-D-xylopyranoside.

The investigations on the species *A. rohituka* W. & A. disclosed the presence of a number of limonoids, sterols, saponins, flavanone and anthraquinone glycosides, di-and triterpenoids, as well as, tetranortriterpenoids have reported in different parts of this plant. An alkaloid has been claimed by Harmon *et al.*, (1979) and named rohitukine as they isolated it from the plant *A. rohituka*. To the best of knowledge, rohitukine is unusual in several ways and it seems to be the frist alkaloid from the *Meliaceae*, because it is the first example of a chromone alkaloid of the flavone alkaloids ficine and isoficine from *Ficus pentoniana, Moraceae*, appear to be the closest analogues of rohitukine observed so far. The presence of the 4-substituted 3-hydroxypiperidine moiety in rohitukine is unusual in a natural compound, anatalline, from *Nicotiana tabacum* and gentialutin, from, *Gentiana asclepiada* being the only similar alkaloids which have come to our attention.

Concerning the naming of this compound the authors probably did not pay attention to Connolly *et al.*, 1976 who isolated and determined a limonoid from the same species and named rohitukin, which derived from the specific name of *A. rohituka*. 'rohitukine' and 'rohitukin' is strikingly similar in hearing, while the compounds are not the same, rather two different phenomena comprising totally different molecular structures and functions. The alkaloid rahitukine was derived from the ethanol extract of the leaves and bark compost but the limonoid rohitukin was from the seeds. It is reported by the authors that the alkaloid rohitukine has no activity on microorganisms.

From the seeds of *Aphanamixis grandifolia,* a close related species of *A. rohituka*. one limonoid amoorastatin has been isolated which showed a greater cell growth inhibition reported by Polonsky *et al.*, (1978a). In the following year (1979) they reported

another limonoid from the same source which is isomeric with that of amoorastatin and named amoorastatone (structure l, Chart-3). This isomer is also cytotoxic.

The major toxic constituent of the species *A. rohituka* aphanamixin (structure e, Chart-2), a triterpene is reported firstly by Chatterjee and Kundu, (1967) from the fruit-shell extract, but Srivastava and Agnihotri claimed in 1984 that it is a product from the seeds, while in the same year Nishizawa and his co-workers furnished the same compound which is said to have the activity on fish LC_{50} 0.06 ppm (48h) (Pettit, *et al.*, 1983). Their reported compound was the very one reported by Chatterjee and Kundu, (1967) and they indicated the term 'fruit-shell' as 'fruit-peel' which in this investigation mentioned as 'fruit-pericarp'.

The minor toxic components named as aphanamols I and II (structure n,o, Chart-3) isolated from the seeds of the related species *A. grandifolia* shown activity on fish LC_{50} 28 ppm (48h) and LC_{50} 27 ppm (48h) were colourless oils and were hydroazulene type sesquiterperoids (Nishizawa *et al.*, 1984).

Only these authors and Polonsky *et al.*, (1978a,b) have been carried out bioassay directed fractionation to afford some bio-active principles from the seeds and fruit-shells of *A. grandifolia* and *A. polystachya*, but it is a very much insufficient volume for the total investigation. And this is why this step has been taken to focus the presence of bio-active components in different parts of *A. rohituka* and to carry on bio-assay directed fractionation to find any active principle not yet reported.

Since the tests carried out on the red flour beetle *T. castaneum* offered unsatisfactory results we have selected some other agents to conduct further experiments to make the findings more conspicuous and complementary. *A. rohituka*, being a medicinal plant was heavily used, and now we have clear conception to comment on the

actions of the constituents of different parts of the plant concerning the past scripts and also for the future possibilities with the title plant.

The activities established by other bio-autographic assessments gave complete sense of bio-active potentialities of the plant and induced us to go through further investigation by fractionation to isolate biologically active compounds. The leaf extracts collected in Pet. spt. found active on *A. aegypti, B. glabrata, C. cucumerinum, C. albicans* and *B. subtilis* while MeOH extracts of the leaves active on *C. albicans* and *B. subtilis*. Pet. spt. extract yielded aphanamixol (compound 2) (structure a, Chart-2), with the molecular formula $C_{20}H_{36}O_2$. But the previous workers mentioned no report on its biological activities, it was found probably the same compound active on both *C. albicans* and *B. subtilis*. The first alkaloid of the family *Meliaceae* rohitukine was also reported from the leaf and stem-bark extract collected in EtOH. Pet. spt. and EtOAc extracts of seeds were active on *C. albicans*. A limonin compound rohitukin, $C_{34}H_{42}O_{13}$ has been isolated from seeds by Connolly *et al.,* (1976). The saponin, stigmasta-5,24(28)-dien-3-*β*-*O*-*β*-D-glucopyranosyl-0-α-L-rhamnopyranoside, (Bhatt *et al.,* 1981) from the seeds extracted by EtOH (95%) and poured into distilled H_2O (500 ml) with continuous stirring and extracting the water soluble portion with C_6H_6. From a very close related species *A. grandifolia* Br. two highly cytotoxic limonoids aphanastatin and amoorastatin were isolated by (Polonsky *et al.,* 1979) and they were found strong to inhibit growth of Murine P-388 lymphocytic leukemia cell line.

Pet. spt., EtOAc and acetone extracts of fruit-pericarp were active on *A. aegypti,* and Pet. spt. extract was active on *C. albicans*. The major toxic component of *A. polystachya* was a known triterpenoid, aphanamixin, $C_{32}H_{50}O_5$ from the petrol-extract of the fruit-shell (Chatterjee and Kundu, 1967, Chatterjee *et al.*, 1970). Aphanamixin and

two other minor toxic principles, aphanamol I and aphanamol II were isolated from the related species *A. grandifolia* fruit-shell.

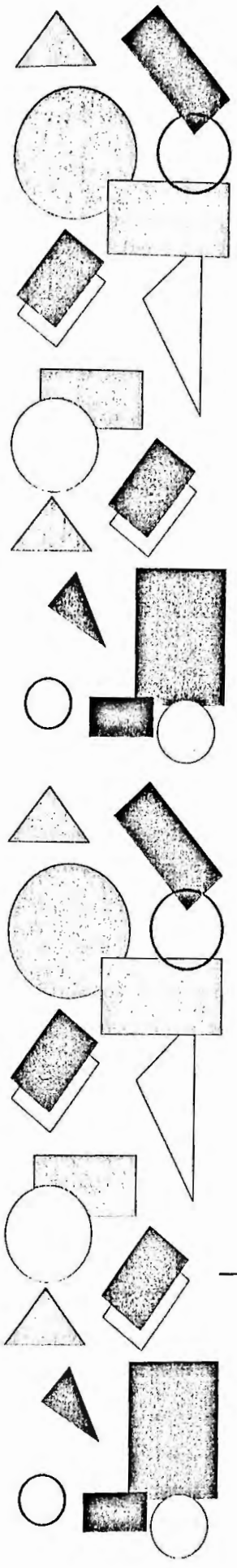
Acetone extract of stem-bark found active on *B. glabrata* even in a lower concentration of 100 ppm, and showed activity on *C. albicans* and *B. subtilis*. MeOH extract found active on all the test agents except *C. albicans*. A triterpenoid, aphanamixinin, has been isolated from the petrol extract. Rectified spirit extract segregated into water soluble and insoluble fractions and the water insoluble material was extracted with Petroleum to yield a β-sitosterol, a stigmasterol ($C_{41}H_{68}O_{10}$) and a saponin, aglaiol-3-O-rhamnosyl-xyloside (Srivastava and Agnihotri, 1984 and 1985) and from the ethanol extract, another saponin poriferasterol-3-rhamnoside, ($C_{35}H_{58}O_5$) was isolated segregating it into water soluble and water insoluble fractions and extracting the water soluble fraction with Pet. ether and benzene respectively indicating no biological activity. From the water insoluble fraction an anthraquinone glycoside 1.5-dihydroxy - 6,7,8-trimethoxy-2-methyl-anthraquinone-3-O-β-D-xylopyranoside and a flavone glycoside naringenin7,4-dimethylether-5-0-α-L-rhamnopyranoside ($C_{23}H_{26}O_9$) (Srivastava and Agnihotri, 1985) was reported. The compound closely related to aphanamixinin, amoorinin (3-di-hydroandirobin) ($C_{27}H_{34}O_7$) was also reported from the stem-bark without indicating its biological activities. The minor toxic components named as aphanamols I and II were also isolated from the seeds of the related species *A. grandifolia*, were hydroazulene type sesquiterperoids and shown activity on fish LC_{50} 28 ppm (48h) and LC_{50} 27 ppm (48h) (Nishizawa *et al.*, 1984).

A flavone glycoside 8-C-methyl-quercetin-3-O-β-D-xylopyranoside (structure h, Chart 2) ($C_{21}H_{20}O_{11}$) from the root extract, indicating no activity, while root extracts collected in Pet. spt. found active on all except *A. aegypti*, in EtOAc found active only on

B. glabrata and in acetone active on *B. glabrata* and *C. albicans*, and no experiment was possible with the MeOH extract as the collections were then missing.

From the tests carried out on *T. castaneum* strains and on other test organisms, viz. *A. aegypti, B. glabrata, C. cucumerinum, C. albicans* and *B. subtilis* we have come to know that the *A. rohituka* extractives are biologically active in different context and the findings by these tests lounced in this investigation the facilitates to find two biologically active constituents, of which one is found similar to formerly reported apanamixol (the compound 2, found active on *C. albicans* and *B. subtilis*), and the other (compound 1, which is strongly active against *C. cucumerinum, C. albicans* and *B. subtilis*) is probably a new natural compound.

The colour bands on the TLC plates revealed with Godin reagent prompted to the presence of a lot of compounds, of which only a little numbers (only about 18) have been reported until now, and this could be a continuous step of going through investigation on the title species *A. rohituka* for more interesting constituents for the promotion of desirable medicines and pesticides for a sustainable environment on the planet.



Conclusion

Conclusion

The family *Meliaceae* comprising several promising tropical species, of which *Amoora rohituka* W. & A. is well known in its region of origin for various folk use and in the traditional medicine. The present investigation carried out by extraction from its different from its different organs, viz. leaves, seeds, fruit-pericarp, stem-bark and roots in four different solvents, viz. petroleum spirit, EtOAc, acetate and MeOH to extract out different types of metabolites. The extracts were evaluated by bioassays made on different strains of the stored grain pest *Tribolium castaneum* which revealed the overall view of the location of biologically active constituents in the plant body. The activity found on all the three strains viz. CR-1, FSS-II and CTC-12 of *T. castaneum* adults and pupae not satisfactory since there was nothing to comment or predict about the plant extractives. There was no distinct scope of showing differences of action. Insecticidal test by topical application on the red flour beetle *T. castaneum* was a phenomenon of control toxicity, which offered mortality in different ratios for different extracts from different parts also showed a phenomenon of recovery. It is essentially a symptom of potentiality of the insect body to prevent unexpected activities of any foreign particle inserted into it.

It was basically a part of a step by step investigation for pesticidal properties:

a. to look for the presence of toxicants, antifeedants, repellents and protectants;
b. to notify the particular location of the bio-active priciples (if present);

c. to find out the type of action and other related nature of the component;
d. to find the way of isolation, purification and characterization of the constituents actually present in the plant body;
e. to develop the procedure of development of the target matter, such as, pesticides or other remedies (if possible); and
f. bring them to the state of public use.

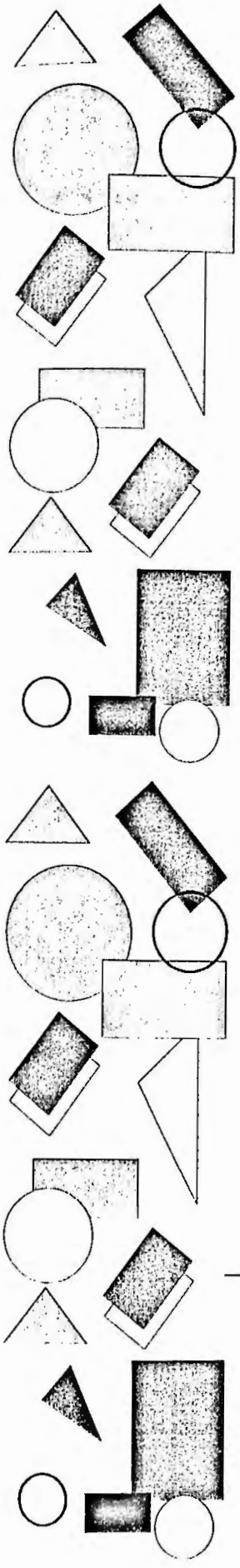
Having successful tests on a single pest it could not be possible to go ahead with the aforesaid proposals. Several other bioassays have also been done to furnish the achievement. Concepts extracted from literature search it influenced to go though some other bioassays on different organisms having different volume of body-size and showing exclusively different nature of damage to human interest. So, the approach covers many parameters of exploration in the field of pest management and disease control. The highest mortality of *T. castaneum* strains was recorded in case of the Pet. spt. extract of the roots, where LD_{50} values were ranging between 0.657 µg/µl (on CR-I pupae for 24 h) to 2.156 µg/µl (on FSS-II pupae for 72 h) and 1.897 µg/µl (on CR-I adult for 24 h) to 7.271 µg/µl (on FSS-II adult for 48 h) and the lowest mortality was observed in case of MeOH extracts of roots, where LD_{50} values were ranging between 34708.590 µg/µl (on FSS-II pupae for 24 h) to 165.147 µg/µl (on FSS-II pupae for 72 h), while the results were more-or-less satisfactory and supposed to do other bioassays. Other bioassays have been done on the mosquito vector of the 'dengue fever' and 'yellow fever' *Aedes aegypti* larvae, schistosomiasis transmitting snails *Biomphalaria glabrata*, plant pathogenic fungi *Cladosporium cucumerinum*, human pathogenic yeast *Candida albicans* and on the human pathogenic bacteria *Bacillus subtilis*. These experiments revealed some interesting information that the plant contains important properties in different location of its body, by which several means of pest control and remedies to combat with diseases could be possible to develop. From the pesticidal context, crude acetone extract showed strong

activity against *B. glabrata* even in a very lower concentration (100 ppm/24 h) and Petroleum spirit extract of the fruit pericarp paid satisfactory action against *A. aegypti*. Especially, these two tests have been made applying the sample dissolved in water. So, the bio-active constituents present in these samples are water soluble substances and of course polar in chromatographic nature.

The strong inhibition zones observed in case of *C. cucumerinum*, while the spores sprayed on the TLC plates run the samples on it and kept in a moist condition to grow the fungi in plenty. EtOAc extract of leaves found to have activity observing clear inhibition zones on *C. cucumerinum* grown sheets.

Being active against *C. cucumerinum* the EtOAc extract enables to conduct activity guided fractionation to yield two pure compounds. After isolation they were tested and both of them were found active against *C. albicans* and *B. subtilis* and one of them showed strong activity against *C. cucumerinum*, which is a colourless sticky substance showed activity in a lower concentration of 10 µg/sopt on the TLC and it is tentatively a new compound with a 14 carbon skeleton.

No doubt, this study revealed an arena new and promising to give emphasis on the title species to lead further study to afford more interesting properties, while only 18 compounds have been reported until now by previous workers and one more constituent going to be added in the list to enhance the expedition of developing natural means of controlling pests, curb diseases and to promote chemotaxonomic activities for the coming generation.



References

References

AGNIHOTRI, V.K., 1987. Poriferasterol-3-rhamnoside, A new saponin from the stem-bark of *Amoora rohituka. Indian Journal of pharmaceutical sciences.* July-August, pp. 149-150.

AGNIHOTRI, V.K., SRIVASTAVA, S.D. and SRIVASTAVA, S.K., 1987a. A new limonoid, amoorinin from the stem-bark of *Amoora rohituka* Wall. *Current science,* August 5, **56** (15): 770-771.

AGNIHOTRI, V.K., SRIVASTAVA, S.D. and SRIVASTAVA, S.K., 1987b. A new limonoid, amoorinin, from the stem-bark of *Amoora rohituka. Planta medica.* pp. 298-299.

AHMED, S.M., CHANDER, H. and PEREIRA, J., 1981. Insecticidal potential and biological activity of Indian indigenous plants against *Musca domestica. Int. Pest Control.* **23**: 170-173.

AINSLIE, J.R., 1937. *A List of Plants Used in Native Medicine in Nigeria.* Imperial Forestry Institute, Oxford Univ. 92 pp.

AMMAN, J., 1736. *Comm. Acad. Scient. imp. Petrop.* **8**: 212.

ANON, 1952. *"The wealth of India."* - a dictionary of Indian raw materials and industrial products. CSIR, New Delhi, **3**: p. 69.

ARBELÁEZ, E.P., 1956. *Plantas Útiles de Columbia,* 3d ed. Bogota, C. Roldan. 831 pp.

ARIGONI, D., BARTON, D.H.R., COREY, E.J. and JEGER, O. in collaboration with CAGLIOTI, L., DEV. S., FERRINI, P.G., GLAZIER, E.R., MELERA, A., PRADHAN, S.K., SCHAFFNER, K., STERNHELL, S., TEMPLETON, J.F. and TOBINAGA, S., 1960. The constitution of limonin. *Experientia.* **16** (2): 41-84.

BACKER, C.A. and BAKHUIZEN VAN DEN BRINK, R.C., 1965. *Flora of Java.* PP. 125.

BANERJI, B. and NIGAM, S.K., 1984. Wood constituents of *Meliaceae*: A review. *Fitoterapia,* **55** (1): 3-36.

BARRY, A.L., 1980. In: *Antibiotics in Laboratory Medicine.* Ed. V., Lorian, Williams and Wilkins, Baltimore/London, pp. 1-26.

BERNAYS, E.A. and CHAPMAN, R.A., 1977. Deterrent chemicals: a basis of oligophagy in *Locusta migratoria* L. *Ecol. Ent.* **2**: 1-18.

BHATT, S.K., SAXENA, V.K. and NIGAM, S.S., 1981. A new saponin from seeds of *Amoora rohituka. Phytochemistry.* **20** (7): 1749-1750.

BROWN, A.W.A., 1968. Insecticide resistance course of age. *Bull. Ent. Soc. Am.* **14**: 3-9.

BUSVINE, J.R., 1971. "*A critical review of the techniques for testing insecticides.*" CAB, London, 345 pp.

CANDOLLE, A.P. de, 1824. *Prodromus Systematis Naturalis Regni Vegetabilis* **1**: 619-626.

CANDOLLE, C. de, 1878. In A. and C. de Candolle, *Monographiae Phanerogamarum.* **1**: 419-758.

CARPENTIER, T.L., NEEL, W.W. and HEDIN, P.A., 1979. A review of host plant resistance of pecan, *Carya illinoensis*, to insect and acarina. *Bull. Ent. Soc. Am.,* **25**: 251-257.

CHANDRASEKHARAN, S. and CHAKRABORTTY, T., 1968. Aphanamixol, a diterpene alcohol from *Aphanamixis polystachya* (Wall.) Parker. *Jour. Indian Chem. Soc.* **45** (3): 208-212.

CHANDRASEKHARAN, S., KUNDU, A.B. and CHAKRABORTTY, T., 1968. Aphanamixinin, a new *seco-ring* β tetranortriterpenoid from *Aphanamixis polystachya* Wall and Parker. *Science and culture* **34** (8): 362-363.

CHATTERJEE, K.K., 1961. Treatment of cancer: a prelude. *Indian Med. Rec. (Calcutta)* **81** (7): 101-105.

CHATTERJEE, A., and KUNDU, A.B., 1967. Isolation, structure and stereochemistry of aphainamixin- a new triterpene from *Aphanamixis polystachya* Wall and Parker. *Tetrahedron letters.* # 16, pp. 1471-1476.

CHATTERJEE, A., KUNDU, A.B., CHAKRABORTTY, T. and CHANDRASEKHARAN S., 1970. Extractives of *Aphanamixis polystachya* Wall (Parker); The structures and stereochemistry of aphanamixin and aphanamixinin. *Tetrahedron.* **26**: 1859-1867.

CHOPRA, R.N., 1933. *Indigenous drugs of India.* Culcutta. The Arts Press. 655 pp.

CHOPRA, R.N., NAYAR, S.L. and CHOPRA, I.C., 1956. *Glossary of Indian Medicinal Plants.* CSIR. pub., New Delhi (India). pp. 21.

CONNOLLY, J.D., OVERTON, K.H. and POLONSKY, J. 1970. The Chemistry and biochemistry of the limonoids and quassinoids. In *Progress in Phytochemistry* (L. Reinhold and Y. Liwschitz, eds.) London: Inter Science Publishers, **2**: 385.

CONNOLLY, J.D., OKORIE, D.A., WIT, L.D. de and TAYLOR, D.A.H., 1976. Structure of Dregeanin and Rohitukin, limonoids from the sub family *Melioideae* of the Family *Meliaceae*. An unusually high absorption frequency for a six-membered lactone ring. *J.C.S. Chem. Comm.*, pp. 909-910.

DAGAR, H.S., 1986. Ethnobotany of the canoe of the Nicobarese tribals. *Indian Forester,* February, pp. 174-178.

DOSKOTCH, R.W., ODELL, T.M. and GODWIN, P.A., 1977. Feeding response of gypsy moth larvae *Lymantria dispar*, to extracts of plant leaves. *Ent.* **6**: 563-566.

DREYER, D.L., 1968. Limonoid bitter principles. In *Progress in the chemistry of organic natural products*. L. Zechmeister, ed., Wien-Springer-Verlag, NY, **26**: 190.

DRURY, H., 1858. *The useful plants of India*. Asylum Press, Madras. 559 pp.

DYTE, C.E., 1970. Insecticide resistance in stored-product insects with special reference to *Tribolium castaneum*. *Trop. stored Prod. Inf.* **20**: 13-15.

DYTE, C.E., and HALLIDAY, D., 1985. Problems of development of resistance to phosphine by insect pests of stored grains. *Bull. Organization Européenne et Mediterranéenne pour la protection des plantes*. **15**: 51-57.

FEINSTEIN, L., 1952. Insecticides from plants. In *Insects: The yearbook of Agriculture*. USDA Washington D.C. pp. 222-229.

FINNEY, D.J., 1947. In *Probit analysis*. Cambridge University Press, London. pp. 333.

FLEMING, J., 1810. *A catalogue of Indian medicinal plants and drugs*. A.H. Hubbard, Calcutta. 72 pp.

GEORGHIOU, G.P. and TAYLOR, C.E., 1977. Pesticide resistance as an evolutionary phenomenon. *Proc. XVth Int. Congr. Ent.* (Washington D.C., 1976), p. 759

GODIN, P., 1954. *Nature*. **154**: 134.

GOLOB, P. and WEBLEY, D.J., 1980. *The use of plants and minerals as traditional protectants of stored products*. Tropical Products Institute. London. 32 pp.

GOTTSTEIN, D., GROSS, D. and LEHMANN, H., 1984. Mikrobiotest mit *Cladosporium cucumerinum* Ell. et Arth. zum Nachweis fungitoxischer Verbindungen auf Dünnschichtplatten. *Arch. Phytopath. u. Pflanzenschutz.* **25**: 111-116.

HARMON, A.D., WEISS, U., and SILVERTON, J.V., 1979. The structure of rohitukine, the main alkaloid of *Amoora rohituka* (syn. *Aphanamixis polystachya*) (Meliaceae). *Tetrahedron letters*. # 8, pp. 721-724.

HARMS, H., 1896. In A. Engler and K. Prantle, *Die Natürlischen Pflanzenfamilien*. **3** (4): 258-308.

HARMS, H., 1940. In A. Engler and K. Prantle, *Die Natürlischen Pflanzenfamilier*. 2nd ed., **19** b1: 1-172.

HARTWELL, J.L., 1970. Plants used against cancer. A survey. *Lloydia* **33** (1-4): 180-182.

HEGNAUER, R., 1969a. *"Chemotaxonomie der Pflanzen."* Birkhäuser verlag Basel and Stuttgart. **5**: 54-70.

HEGNAUER, R., 1969b. *"Chemotaxonomie der Pflanzen"* Birkhäuser verlag Basel and Stuttgart. **9**: 421-428.

HERNÁNDEZ, F., 1942-1946. *Historia de las Plantas de Nueva España*. Mexico, Imprenta Universitaria. 1104 pp.

HEWAT, M.L., 1908. *Bantu folk lore*. Cape Town, T. M. Miller. 112 pp.

HEYDE, J.V.D., SAXENA, R.C. and SCHMUTTERER, H., 1983. Neem oil and Neem extracts as potential insecticides for control of hemipterous rice pests. *Proc. 2nd Int. Neem. Cong. Rauischholzhausen*. pp. 377-390.

HOERNLE, A.F.R., 1893-1912. *The Bower manuscript*. Calcutta, Sup't. Govt. Printing. 401 pp.

HOMANS, A. L. and FUCHS, A., 1970. Direct bio-autography on thin-layer chromatograms as a method for detecting fungi-toxic substances. *J. Chromatogr.* **51**: 327-329.

HOOKER, J.D. 1862. In G. Bentham and J.D. Hooker, *"Genera Plantarum."* **1**: 327-340.

HOSTETTMANN, K., KIZU, H. and TOMIMORI, T., 1982. *Planta Med.* **44**: 34.

HOSTETTMANN, K., HOSTETTMANN, M. and MARSTON, A., 1986. *Preparative chromatographic technics: applications in natural product isolation.* Springer-Verlag, Berlin.

HOSTETTMANN, K., HAMBURGER. M., HOSTETTMANN, M. and MARSTON, A., 1991. New developments in the separation of natural products. In *Modern Phytochemical Methods.* (ed. N.H. FISCHER, M.B. ISMAN, and H.A. STAFFORD), Plenum Press, New York, pp. 1-31.

HOSTETTMANN, K., MARSTON, A. and WOLFENDER, J.-L., 1995. Strategy in the search for new biologically active plant constituents, In *Phytochemistry of plants used in traditional medicine. Proc. Phytochem. Soc. Eur.* **37**: 17-45.

HUFFORD, C.D. and CLARK, A.M., 1988. Discovery and development of new drugs for systematic opportunistic infections. In *Studies in Natural products Chemistry.* (ATTA-UR-RAHMAN, ed.), Elsevier, Amsterdam, **2**: pp. 421-452.

HUXTABLE, R.J., 1992. The pharmacology of extinction. *Journal of Ethnopharmacology.* **37**: 1-11.

IRSHAD. M. and GILLANI, W.A., 1990. Resistance in *Tribolium castaneum* (Herbst) (Coleoptera:Tenebrionidae) against malathion. *Pakistan J. Zool.* **22**: 257-262.

ISLAM, B.N., 1983. Pesticidal action of Neem and certain indigenous plants and weeds of Bangladesh. *Proc. 2nd. Int. Neem. Conf. Rauischholzhausen.* pp. 263-290.

JACOBSON, M., 1977. Isolation and identification of toxic agents from plants. In *Host plants resistance to pests. ACS Symp. Ser.* **62**: 153-164.

JACOBSON, M., REDEERN, R.E. and MILLS, G.D. JR., 1975. Naturally occurring insect growth regulators-II. Screening of insect and plant extracts as insect juvenile hormone mimmics. *Lloydia,* **33**: 455-472.

JAIN, S.A. and SRIVASTAVA, S.K., 1984. Betulin-3β-*O*-β-D- xylopyranoside from the roots of *Amoora rohituka*. *Indian Journal of Pharmaceutical Sciences*. July-August, p-161-162.

JAIN, S.A. and SRIVASTAVA, S.K., 1985. 8-C-methyl-quercetin 3-*O*-β-D-xylopyranoside, A now flavone glycoside from the roots of *Amoora rohituka*. *Journal of Natural Products*. Mar.-Apr., **48** (2): 299-301.

JEFFERIES, P.R. and PAYNE, T.G., 1965. The Chemistry of the Euphorbiaceae. *Aust. J. Chem.* **18**: 1441-1450.

JURD, L. and MANNERS, G.D., 1980. Wood extractives as models for the development of new types of pest control agents. *J. Agric. Fd. Chem.* **28**: 183-188.

JUSSIEU, A.L. de, 1789. *Genera Plantarum*, pp. 263-266.

JUSSIEU. A. de, 1830. Mémoire sur le Groupe de Méliacées. *Mém. Mus. Hist. Nat. Paris.* **19**: 153-304.

KAVIRATNA, A. C., 1909. *Charaka-Samhita*, (1888-1909). Calcutta, Corinthian Press **1-2**: 1718 pp.

KHAN, M.S. and HASSAN, M.A., 1981. Bangladesher Bishakta Udvid. pp. 35.

KHANAM, L.A.M., TALUKDER, D., KHAN, A.R. and RAHMAN, S.M., 1990. Insecticidal properties of royna, *Aphanamixis polystachya* Wall. (Parker) (*Meliaceae*) against *Tribolium confusum* Duval. *J. Asiat. Soc. Bangladesh Sci.* **16**: 71-74.

KIRTIKAR, K.R. and BASU, B.D., 1935. *Indian Medicinal Plants*. Lalit Mohan Basu Pub. Allahabad (India), **1**: 551.

KOSTELETZKY, B.F., 1836. *Allgemeine medizinisch-pharmazeutische Flora*, (1831-1836). Prague, Borrosch and Andre, **1-6**: 2237 pp.

KOSTERMANS, A.J.G.H., 1982a. Notes on Ceylonese plants II. *Acta. Bot. Neerl.* February, **31** (1/2): p. 121-132.

KOSTERMANS, A.J.G.H., 1982b. A new *Amoora* (*Meliaceae*) from India. *Acta. Bot. Neerl.* February, **31** (1/2): 133.

KOSTERMANS, A.J.G.H., 1982c. The Genus *Dysoxylum* (Meliaceae) in Cylon. *Acta. Bot. Neerl.* August, **31** (4): p. 321-326.

LIDERT, Z., TAYLOR, D.A.H. and THIRUGNANAM, M., 1985. Insect antifeedant activity of four prieurianin-type limonoids. *Journal of Natural products.* **48** (5): 843-845.

LOUREIRO, J. de, 1790. *Flora Cochinchinensis.* Lisbon (Ulyssipone). 745 pp.

MABBERLEY, D.J., 1982. Notes on Malesian *Meliaceae* for, *'The Tree Flora of Malaya'. The malaysian Forester.* **45** (3): 448-455.

MARSTON, A. and HOSTETTMANN, K., 1991. Modern separation methods. *Natural Product Reports,* **8**: 391-423.

MARTIUS, C.F.P. de, 1843. *Systema Materiae Medicae Vegetabilis Brasiliensis.* Leipzig and Vienna. 155 pp.

MENN, J.J., 1980. Contemporary frontiers in chemical pesticide research. *J. Agric. Fd. Chem.* **28**: 2-8.

MITSCHER, L.A. and RAO, R.G.S., 1984. The search for new antimicrobial agents: unusual sources. In *Natural products and drug development* (ed. P. Krogsgaard-Larsen, L. Brogger-Christensen, and H. Kofod). pp. 193-209. Munksgaard, Copenhehen.

MUNAKATA, K., 1970. Insect antifeedant in Plant. In WOOD, D. L. SILVERSTEIN, P. M. and NAKAZIMA, M. (Eds.) *Control of Insect Behaviour by Natural Products. Academic Press,* NY. pp-179-187.

NAIK, R.G., KATTIGE, S.L., BHAT, S.V., ALREJA, B., SOUZA, N.J. de and RUPP, R.H., 1988. An antiflammatory cum immunomodulatory piperidinylbenzopyranone from *Dysoxylum binectariferum*: isolation, structure and total synthesis. *Tetrahedron* **44** (7): 2081-2086.

NISHIZAWA, M., INOUE, A., HAYASHI, Y., SASTRAPRADJA, S., KOSELA, S. and IWASHITA, T., 1984. Structure of aphanamol I and II. *J. Org. Chem.* **49**: 3660-3662.

O'SHAUGHNESSY, W.B., 1841. *The Bengal dispensary and pharmacopoeia.* Calcutta, Bishop's College Press. 794 pp.

PARK, T., 1962. Beetles, competition and population. *Science* **138**: 1369-1375.

PARK, T., and FRANK, M.B., 1948. The fecundity and development of the flour beetles, *Tribolium castaneum* and *Tribolium confusum* at three constant temperatures. *Ecology*, **29**: 368-375.

PASALU, I.C. and BHATIA, S.K., 1983. Inheritance of resistance to malathion in *Tribolium castaneum* (Herbest). *Proc. Indian Acad. Sci. (Anim. Sci.).* **92**: 409-414.

PENNINGTON, T.D. and STYLES, B.T., 1975. A generic monograph of the *Meliaceae. Blumea* **22** (3): 419-540.

PETTIT, G.R., BARTON, D.H.R., HERALD, C.L., POLONSKY, J., SCHMIDT, J.M. and CONNOLLY. J.D., 1983. Evaluation of limonoids against the Murine P388 lymphocytic leukemia cell line. *Journal of Natural Products.* May-Jun., **46** (3): 379-390.

PIMENTAL, D., 1977. Ecological basis of insect pest, pathogen and weed problems. In: CHERRETTE, S.M. and SAGAR, G.R. (Eds.) *Origin of Pest, Parasites, Disease and weed Problems.* Blackwells Scientific Publications, Oxford, U.K. p. 3.

PIMENTAL, D., 1981. An overview of integrated pest management. Department of Ecology and systematics, Cornell University, Ithaca, NY (mimeo., 52 pp).

PIMENTAL, D., ANDOW, D., DYSON-HUDSON, R., GALLAHAN, D., JACOBSON, S., IRISH, M., SHEPARD, M., THOMPSON, T., and VINZANT, B., 1980. *Environmental and social costs of pesticides: a preliminary assessment. Oikos*, **34**: 125-140.

POLONSKY, J., VARON, Z., ARNOUX, B., PASCARD, C., PETTIT, G.R. and SCHMIDT, J.M., LANGE, L.M., 1978a. Isolation structure of aphanastatin. *Journal of the American Chemical Society.* April 12, **100** (8): pp. 2575-2576.

POLONSKY, J., VARON, Z., ARNOUX, B., PASCARD, C., PETTIT, G.R. and SCHMIDT, J.M., 1978b. Isolation structure of amoorastatin. *Journal of the American Chemical Society.* November 22, **100** (24): pp. 7731-7733.

POLONSKY, J., VARON, Z., MARAZANO, Ch., ARNOUX, B., PETTIT, G.R., SCHMIDT, J.M., OCHI, M. and KOTSUKI, H., 1979. The structure of amoorastatone and the cytotoxic limonoid 12-hydroxyamoorastatin. *Experientia.* **35** (8): 987-989.

POMPA, G., 1875. *Coleccion de Medicamentos Indigenas, 5th ed.* Caracas, Rójas Hermanos. 175 pp.

RAHALISON, L., 1994. *Mise au Point et application d'une methode de depistage d'activité, antifongique (Candida albicans) dans des extraits végétaux.* Ph.D. Thesis, Univeristy of Lausanne, Switzerland.

RAHALISON. L., HAMBURGER, M., HOSTETTMANN, K., MONOD, M. and FRENK, E., 1991. A bio-autographic agar overlay method for the detection of antifungal compounds from higher plants. *Phytchemical analysis,* **2**: 199-203.

RAHALISON, L., HAMBURGER, M., HOSTETTMANN, K., MONOD, M., FRENK, E., GUPTA, M.P., SANTANA, A.I., CORREA A., M.D. and GONZALEZ, A.G., 1993. Screening for antifungal activity of Panamanian plants. *Int. J. Pharmacog.* **31** (1): 68-76.

RAZZAQUE, M.A., SAYEED, M. and DAS, S.C., 1983. Vegetable oil from fruits of forest species. *Bano Biggyan Patrika,* Jan-July, **12** (1 and 2): pp 22-29.

ROJATKAR, S.R., CHIPLUNKAR, Y.G. and NAGASAMPAGI, B.A., 1994. A diterpene from *Cipadessa fruticosa* and *Grangea maderaspatana. Phytochemistry,* **37** (4): 1213-1214.

ROSENTHAL, D.A., 1861. *Synopsis Plantarum diaphoricarum.* Erlangen, F. Enk. 1359 pp.

SAXENA, R.C., 1983. Naturally occurring pesticides and their potential. In: *Chemistry and World food supplies: The New Frontiers.* Pergamon Press, Oxford, New York, 143-161.

SCHMUTTERER, H., 1981. Ten years of Neem research in the Federal Republic of Germany. *Proc. Ist. Int. Neem Conf.* (Rollach-Egern, 1980). pp. 21-32.

SHUDHAKAR, T,R., PANDEY, N.D. and TEWARI, G.C., 1978. Antifeeding property of some indigenous plants against mustard saw fly, *Athalia proxima* (Hymenoptera: Tenthredinidae). *Indian J. Agric. Sci.*, **48**: 16-18.

SRIVASTAVA, S.K. and AGNIHOTRI, V. K., 1984. Chemical investigation of the stem-bark of *Aphananixis polystachya. Current Science.* December 20, **53** (24): 1288-1289.

SRIVASTAVA, S.K. and AGNIHOTRI, V.K., 1985. New Glycosides from the stem-bark of *Aphananixis polystachya. Current Science,* January 5, **54** (1): 38-40.

SUKUMARAN, D. PARASHAR, B.D. and RAO, K.M., 1994. Moluuscicidal properties of *Agave americana* and *Balanites aegyptica. Int. J. Pharmacog.*, **32**(3): pp. 232-238.

SUNDARRAO, K., BURROWS, I., KUDUK, M., YI, Y.D., CHUNG, M.H., SUH, N.J. and CHANG, I.-M., 1993. Preliminary screening of antibacterial and antitumor activities of Papua New Guinean native medicinal plants. *Int. J. Pharmacogn.* **31** (1): 3-6.

TALUKDER, F.A. and HOWSE, P.E., 1994. Repellent., toxic and food protectant effect of Pithraj, *Aphananixis polystachya* extracts against pulse beetle, *Callosobruchus chinensis* in storage. *J. Chem. Ecol.* **20**: 899-908.

TALUKDER, F.A. and HOWSE, P.E., 1995. Evaluation of *Aphananixis polystachya* as a source of repellents, antifeedants, toxicants and protectants in storage against *Tribolium castaneum* (Herbst). *J. Stored. Prod. Res.* **31** (1): 55-61.

WAISS, A.C.Jr., CHEN, B.G., ELLIGER, C.A., DREYER, D.L., BINDER, R.G. and GUELDNER, R.C., 1981. Insect growth inhibitor in crop plants. *Bull. Ent. Soc. Am.*, **27**: 217-221.

WARTHEN, J.D.Jr., 1979. *Azadirachta indica:* A source of insect feeding inhibitors and growth regulators. *U.S. Dep. Agric. Rev. Man.*, ARM-NE-4

WATT, G., 1889. *A dictionary of the economic products of India* (2nd reprint 1972). International book distributors, Dehradun, India, pp. 223.

WATT, J.M. and BREYER-BRANDWIJK, M.G., 1962. *Medicinal and poisonous plants of southern and eastern Africa.* 2nd ed. Edinburgh and London, E. and S. Livingstone Ltd. 1457 pp.

WILLIS, J.C., 1966. *A dictionary of flowering plants and ferns.* 7th ed. (Revised by H.K. Airshaw), Cambridge University Press, Cambridge, p. 709.

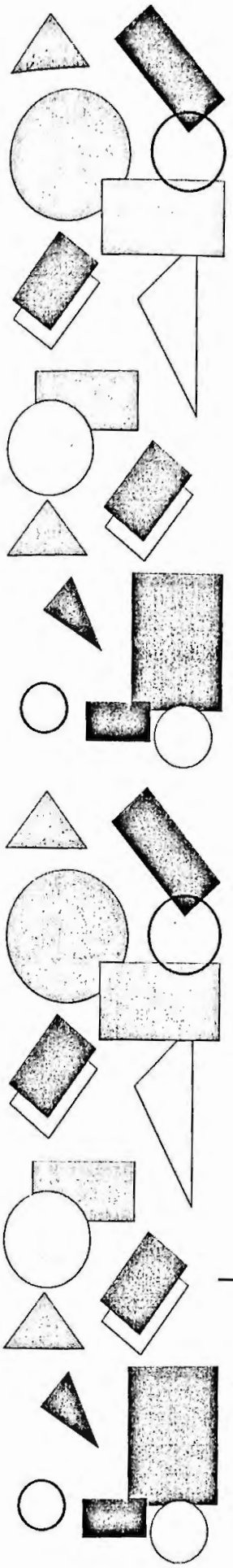
XIMENEZ, F., 1888. *Cuatro Libros de la Naturaleza y Virtudes de las Plantas y Animales, de Uso Medicinal en la Nueva España.* Of tip de la secr. de fom. Mexico. 342 pp.

ZANI, C.L., MARSTON, A., HAMBURGER, M. and HOSTETTMANN, K., 1993. Molluscicidal milliamines from *Euphorbia milli* var. *hislopii. Phytochemistry,* **34** (1): pp. 89-95.

ZETTLER, J.L., 1991. Pesticide resistance in *Tribolium castaneum* and *Tribolium confusum* (Coleoptera:Tenebrionidae) from flour mills in the USA. *J. Econ. Ent.* **84**: 763-767.

ZETTLER, J.L. and CUPERUS, G.W., 1990. Pesticide resistance in *Tribolium castaneum* (Coleoptera:Tenebrionidae) and *Rhizopertha dominica* (Coleoptera:Bostrichidae) in wheat. *J. Econ. Ent.* **83**: 1677-1681.

ZYROMSKA-RUDZKA, H., 1966. Abundance and emigration of *Tribolium* in laboratory MODEL. EKOL. POLE. A. 14 : 491-518.



Abstract

Abstract

The *Meliaceae* is a botanical family confined to tropical and subtropical areas with a distribution from the rain forest to the mangrove swamps and semi-deserts. Many species of this family are used as drugs and in traditional medicine. The plant chosen for study here, *A. rohituka* W. & A., a tree species is native to Southeast Asia, East Asia and Australia. The present work deals with the phytochemical investigation of five different materials (leaves, seeds, fruit-pericarp, stem-bark and roots of the plant). All air dried materials were extracted successively with Petroleum spirit, EtOAc, acetone and MeOH. The title experiments were done on different strains of the stored grain pest *Tribolium castaneum*. The highest mortality was recorded in case of the Pet. spt. extract of the roots, where LD_{50} values were ranging between 0.657 µg/µl (on CR-I pupae for 24 h) to 2.156 µg/µl (on FSS-II pupae for 72 h) and 1.897 µg/µl (on CR-I adult for 24 h) to 7.271 µg/µl (on FSS-II adult for 48 h) and the lowest mortality was observed in case of MeOH extracts of roots, where LD_{50} values were ranging between 34708.590 µg/µl (on FSS-II pupae for 24 h) to 165.147 µg/µl (on FSS-II pupae for 72 h), while the results were more-or-less satisfactory and supposed to do other bioassays. Whereas, the EtOAc extracts of leaves showed activity against the Gram-positive bacterium *Bacillus subtilis*, the human

pathogenic yeast *Candida albicans*, the plant pathogenic fungus *Cladosporium cucumerinum*, the yellow fever and Dengue vector mosquito *Aedes aegypti* larvae and the schistosomiasis transmitting snail *Biomphalaria glabrata* in bio-autographic assays. Activity guided fractionation by a combination of silica-gel column chromatography, gel filtration on sephadex LH20 and low-pressure column chromatography provided two active natural compounds: compound-**1** '4-hydroxy-5-methyl-undecadienedione' and compound-**2** as 'aphanamixol'

Beside furnishing chemotaxonomic data on the plant family *Meliaceae*, these investigations have led to the characterization of two compounds with interesting biological activities. The first one is active against *C. cucumerinum*, *B. subtilis* and *C. albicans* and it showed strong activity against *C. cucumerinum* even in a very lower concentration of 1 ppm, i.e., 10 µg/1µl/spot and it is tentatively a new compound, and the second one is active against *B. subtilis* and *C. albicans*, which is a known compound reported earlier for the same plant. Spectroscopic methods (^{1}H- and ^{13}C-NMR, MS and IR) allowed the characterization of 2 compounds, one of which is tentatively a new natural product with remarkable biological activities.

Résumé

Les Méliacées forment une famille botanique, exclusivement tropicale et sous-tropicale avec une destribution dans la foret tropicale humide jusca au marais de mangrove et sous désert. Plusieurs espèces de cette famille sont utilisées comme medicament et en médecine traditionnelle. *Amoora rohituka* W. & A. choisy est un arbs native de la région sud-est de l'Asie, est de l'Asie et de l'Australie. Le présent travail a porté sur les cinq organes different (des feuilles, des graines, des péricarpes de fruits, des ecorces et des racines) pour les investigations phytochimiques. Aprés séchage, tous les organes a été extrait avec des solvants de polarité croissante, à savoir l'spirit de pétrole (Pet. spt.), l'acetate d'ethyle (EtOAc), l'acetone et le méthanol (MeOH). Les expériences du titre a fait sur les variété différences du nuisibles de céréales mettre un réserve, *Tribolium castaneum*, La mortalité sur des extraites Pet. spt. de racines, et des valeurs LD_{50} a été au milieu 0.657 µg/µl (sur CR-1 pupae pour 24 h) à 2.156 µg/µl (sur FSS-II pupae pour 72 h) et 1.897 µg/µl (sur CR-1 adult pour 24 h) à 7.271 µg/µl (sur FSS-II adult pour 48 h); et la mortalité bas aussi sur des extraites méthanoliques de racines, et des valeurs LD_{50} a été au milieu 34708.590 µg/µl (sur FSS-II pupae pour 24 h) à 165.147 µg/µl (sur FSS-II pupae pour 72 h); et aussi il y a des guérisons de insectes; mais des résultats son moins satisfaisantes et insister pour faire encore les tests biologiques. Mais,

l'extrait acetat d'ethyl a présenté des activité d'inhibition de la croissance de la bactérie Gram-positive *Bacillus subtilis*. L'activité antifongique a été mesurée en observant l'inhibition de la croissance de l'yeast human pathogène *Candida albicans* et en observant l'inhibition de la croissance du champignon phytopathogène *Cladosporium cucumerinum;* Cette extrait a démontré une activité larvircide contre le moustique *Aedes aegypti* une vector de yellow fever et Dengue, et aussi démontré une activité molluscicide contre le escargot *Biomphalaria glabrata,*une agent de la transmission de schistosomiasis dans les testes bioautographiques.

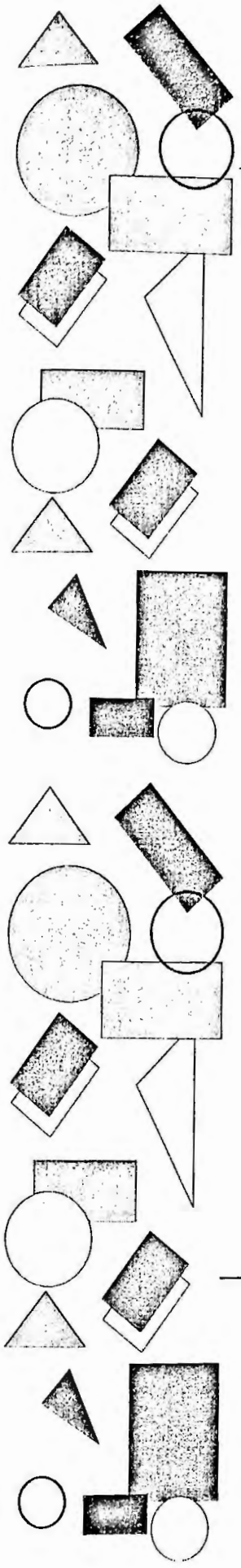
Son fractionnement guide par activité par une combination de chromatographie sur colonne flash de gel de silice, filtration sur gel sephadex LH20 et chromatographie liquide à basse pression a fourni les deux produits naturels, composé **1**. 4-hydroxy-5-methyl-undecadienedione et composé **2.** aphanamixol, un composé connu et déjà rapporté sur la même espèce. Outre de mombreuses indication sur les caractéristiques chimiotaxonomiques des méliacées, nos recherches ont permis de caractériser deux composés dont quelquesuns sort dotés d'activités biologique intéressantes. la première composé a montré les activité contré la bactérie *B. subtilis,* le champignon *C. albicans* et plus contré le champignon *C. cucumerinum*, avec une concentration 1 ppm, i.e., 10 µg/µl/spot et provisoirement c'est une nouveau produit naturel, mais la deuxième composé dans les deux que a isolée a été actif contre la bactérie Gram-positive *B. subtilis* et contre le champignon *C. albicans*. Les méthods spéctroscopiques (^{1}H-et ^{13}C-NMR, MS et IR) ont permis tentativement la caractérisation de deux composés ont une (la deuxiéme) ont le nouveaux produit naturel avec des activité biologiques rémarquables.

সার-সংক্ষেপ

Meliaceae উদ্ভিদ পরিবারের উদ্ভিদগুলো ক্রান্তিয় ও উপক্রান্তিয় অঞ্চলে, সাধারণতঃ রেইন ফরেস্ট বনভূমি থেকে ম্যানগ্রোভ জলাভূমি বা উপমরুভূমি অবধি বিস্তৃত। এ পরিবারের অনেক প্রজাতিই ঔষধ উৎপাদনে ও লোক চিকিৎসায় ব্যবহৃত হয়। নির্বাচিত *Amoora rohituka* W. & A. দক্ষিণ-পূর্ব এশিয়া, পূর্ব-এশিয়া ও অষ্ট্রেলিয়ায় পাওয়া যায়। বর্তমান গবেষণা কাজটি এই গাছটির বিভিন্ন অংগের (পাতা, বীজ, ফলত্বক, বাকল ও মূল) জৈব রাসায়নিক উপাদানের বিশ্লেষণ নিয়ে। বাতাসে শুকানো উপাদান থেকে পেট্রোলিয়াম স্পিরিট, ইথাইল অ্যাসিটেট, অ্যাসিটোন ও মিথানলে নির্যাস সংগ্রহ করা হয়। গবেষণা শিরোনামে সম্পৃক্ত পরীক্ষণগুলি গুদামজাত শস্যের ক্ষতিকারক পোকা *Tribolium castaneum* এর ওপর করা হয়। এ পরীক্ষণে উচ্চ মৃত্যুহার পাওয়া গেছে মূলের পেট্রোলিয়াম স্পিরিট নির্যাসে যেখানে LD_{50} ছিল ০.৬৫৭ µg/µl (CR-I মুককীটে ২৪ ঘন্টায়) থেকে ২.১৫৬ µg/µl (FSS-II মুককীটে ৭২ ঘন্টায়) ও ১.৮৯৭ µg/µl (CR-I পূর্ণাংগ পোকায় ২৪ ঘন্টায়) থেকে ৭.২৭১ µg/µl (FSS-II পূর্ণাংগ পোকায় ৪৮ ঘন্টায়) এর মধ্যে এবং নিম্ন মৃত্যুহারও পাওয়া গেছে মূলের মিথানল নির্যাসে, যেখানে LD_{50} ছিল ৩৪৭০৮.৫৯০ µg/µl (FSS-II মুককীটে ২৪ ঘন্টায়) থেকে ১৬৫.১৪৭ µg/µl (FSS-II মুককীটে ৭২ ঘন্টায়) এর মধ্যে; আর কিছু কিছু ক্ষেত্রে পুনরুজ্জীবিত হওয়ার ঘটনাও ঘটেছে এবং এতে ফলাফলগুলো মোটের ওপর আশাব্যঞ্জক ছিল, যা অন্যান্য পরীক্ষণ পরিচালনার প্রয়োজনীয়তা তুলে ধরে। অবশ্য, ইথাইল অ্যাসিটেটে সংগৃহিত নির্যাসটি গ্রাম পজিটিভ ব্যাক্টেরিয়া *Bacillus subtilis*, মানবদেহে রোগ সংক্রামণকারী ঈষ্ট *Candida albicans*, উদ্ভিদদেহে রোগ সংক্রমণকারী ছত্রাক *Cladosporium cucumerinum*, পীতজ্বর ও ডেঙ্গু রোগের বাহক মশা *Aedes aegypti*-এর লার্ভা ও সিস্টোসোমিয়াসিস রোগের বাহক শামুক *Biomphalaria glabrata*-র উপর পরিচালিত পরীক্ষায় অনুজীব-বিধ্বংসী

কার্যকলাপ দেখিয়েছে। কার্যকারিতা পর্যবেক্ষণ সহযোগে পৃথকীকরণ প্রক্রিয়ায় সিলিকা-জেল কলাম ক্রোম্যাটোগ্রাফী, সেফাডেক্স LH20 সহযোগে জেলপরিস্রাবণ ও লো-প্রেসার কলাম ক্রোম্যাটোগ্রাফী ব্যবহারে দু'টি অনুজীব-বিধ্বংসী প্রাকৃতিক উপাদান (১. 4-hydroxy-5-methyl-undecadienedione ও ২. aphanamixol) পাওয়া গেছে।

Meliaceae পরিবারের রাসায়নিক শ্রেণীবিন্যাস-উপাত্তে তথ্য প্রদানের পাশাপাশি আমাদের এই গবেষণা কার্যক্রম দু'টি অনুজীব-বিধ্বংসী প্রাকৃতিক যৌগের গঠন কাঠামো উপহার দিয়েছে। এ দু'টি যৌগের প্রথমটি (4-hydroxy -5-methyl-undecadienedione) *B. subtilis* ও *C. albicans* ছাড়াও *C. cucumerinum* এর বিরুদ্ধে অতিসামান্য মাত্রায়ও (যেমন 1 ppm. বা 10 µg/1µl/spot) কার্যকারিতা দেখাচ্ছে এবং উল্লেখ্য যে, প্রাথমিক বিবেচনায় এটি একটি নতুন প্রাকৃতিক অনুজীব বিধ্বংসী যৌগ। আর দ্বিতীয়টি (aphanamixol) *B. subtilis* ও *C. albicans*-এর বিরুদ্ধে কার্যকারিতা দেখায়। এক্ষেত্রে স্পেক্ট্রোস্কোপি পদ্ধতিসমূহের (^{1}H-ও ^{13}C-NMR, MS ও IR) ব্যবহারের মাধ্যমে এ দু'টি উপাদানের গঠন কাঠামো নিশ্চিত করা হয়েছে যার ১টি অত্যন্ত সক্রীয় অনুজীব-বিধ্বংসী একটি নতুন প্রাকৃতিক যৌগ।



Appendices

To conduct probit calculation the following indications are followed:

1. Data sheets were prepared as nondocument files (pressing 'N') on a Word Star programme on IBM compatible computer and saved pressing 'X';
2. GWBASIC opened, 'F3' pressed to load PROBIT programme;
3. 'F2' pressed to run the programme and input file name was given when demanded, and similarly a output file name also.
4. The output files then saved on Wordperfect programme to convert into desirable format before printing out.

Program list of Probit analysis:

```
5 INPUT "ENTER INPUT FILE NAME "; A$
10 OPEN "I" ,3,A$
20 GOSUB 1120
25 INPUT "ENTER OUTPUT FILE NAME " ; B$
30 OPEN "O" ,2, B$
35 INPUT # 3, QW
40 INPUT # 3 , D
50 FOR I=1 TO D
60 INPUT#3 ,DS( I ) , N( I )
70 NEXT I
75 FOR I TA =1 TO QW
80 ON ERROR GOTO 1810
90 CLS
100 'IF D> 10 THEN GOTO 130:IF D<3 THEN GOTO 130
110 FOR I = 1 TO D
```

```
120 INPUT#3, K( I )
130 NEXT I
140 FOR I = 1 TO D : PRINT I ; K( I ) : NEXT
240 FOR I = 1 TO D
250 P( I ) = K( I )*100/N( I )
260 NEXT I
280 INPUT#3, CN, CK
330 C=( CK*100) /CN
340 MN=0 : J%=1
350 FOR I=1 TO D
360 PC( I )=( P( I )-C )/( 100-C ) : PC( I )=PC( I )*100
370 DL ( I )=LOG( DS( I ) )* .43429
380 IF DL ( I ) <MN THEN MN=DL( I )
390 DP=PC( I )-INT(PC( I ))
400 IF DP>=.5 THEN PC( I )=INT(PC( I ))+1
410 IF DP< .5 THEN PC( I )=INT(PC( I ))
420 EP( I )=TB( PC( I ))
430 NEXT I
440 IF MN<O THEN UN=INT( MN )
450 FOR I=1 TO D:DL( I )=DL( I )-UN
460 NEXT I
470 SX=O : SY=O : X2=O : Y2=O : XY=0
480 FOR I=1 TO D
490 SX=SX+DL( I ) : X2=X2+( DL( I )^2)
500 SY=SY+EP( I ) : Y2=Y2+(EP( I)^2 )
510 XY=XY+DL( I )*EP( I )
520 NEXT I
530 CX=X2-( ( SX^2 )/D )
540 CY=Y2-( ( SY^2 )/D )
550 CR=XY-( ( SX*SY )/D )
560 B=CR/CX
570 A=(SY/D )-( B*(SX/D ) )
580 MX=SX/D : MY=SY/D
```

```
590 LD=MX+( ( 5-MY )/B )
600 COSUB 1740 : REM PRINOUT
610 SX=0 : SY=0 : X2=0 : Y2=0 : XY=0 : SW=0
620 J%=J%+1
630 FOR I=1 TO D
640 EY( I )=( LD·( I )*B ) +A
650 EZ=INT( EY ( I )*10 )
660 WP( I )=TC( EZ, 1 )+TC( EZ, 2 )*PC( I )
670  WC=TC(EZ ,3 )
680 WT( I )=N( I )*WC
690 SW=SW+WT ( I )
700 SX=SX+WT( I )*DL( I )
710 SY=SY+WT( I )*WP( I )
720 X2=X2+WT(  I )* LD( I ) *LD ( I )
730 Y2=Y2+WT( I )*WP( I )*WP( I )
740 XY=XY+WT( I )*DL( I )*WP( I )
750 NEXT I
760 MX=SX/SW
770 MY=SY/SW
780 B=( XY-( MX*SY ) )/( X2-( MX*SX ) )
790 A=MY-B*MX
800 LD=MX+( 5-MY )/B
810 GOSUB 1740 : REM PRINT OUT
820 DF=O : MD=O
830 FOR I=1 TO D
840 FP( I )=( DL( I )*B )+A
850 DF=ABS( FP( I )-EY( I ) )
860 IF MD<DF THEN MD=DF
870 NEXT I
880 GOSUB 1620 : REM PRINT TABLE
890 IF MD>=.1 THEN GOTO 610 : REM REPEAT ANOTHER CYCLE
900 V=( LD-MX )^2
910 V=V/( X2-MX*SX )
```

```
920 V=V+1/SW
930 V=V/( B*B )
940 CH=( Y2-MY*SY )-B*( XY-MX*SY )
950 DF=INT( D-2 )
960 PRINT "CHI-SQUARED IS ";CH;" WITH ";DF;" DEGREES OF FREEDOM"
965 PRINT#2, "CHI-SQUARED IS ";CH;" WITH ";DF;" DEGREES OF FREEDOM"
970 CK=AT( DF )
980 IF CH<CK THEN GOTO 1010
990 V=V*( CH/DF )
1000 PRINT " VARIANCE HAS BEEN ADJUSTED FOR HETEROGENEITY"
1005 PRINT#2, "VARIANCE HAS BEEN ADJUSTED FOR HETEROGENEITY"
1010 SE=SQR( V)
1020 IF CH<CK THEN PRINT "NO SIG HETEROGENEITY"
1025 IF CH<CK THEN PRINT#2, "NO SIG HETEROGENEITY"
1030 L1=LD-1, 96*SE : L2=LD+1, 96*SE
1040 PRINT "LOG LD-50 IS "; LD
1045 PRINT#2, "LOG LD-50 IS ";LD
1050 PRINT "LD-50 IS "; 10^( UN+LD )
1055 PRINT#2, "LD-50 IS ";10^( UN+LD )
1060 PRINT "95% CONF LIMITS ARE ";10^( UN+L1 ); " TO ";10^( UN+L2)
1065 PRINT#2, "95% CONF LIMITS ARE ";10^(UN+L1 )' " TO "'10^( UN+L2 )
1100 PRINT : PRINT :PRINT#2, :PRINT#2,
1105 NEXT ITA
1110 END
1120 DIM TB( 99 ): REM TABLES
1130 FOR I = 1 TO 99
1140 READ TB( I )
1150 DATA 2.67 ,2.95 ,3.12 ,3.25 ,3.36 ,3.45 ,3.52 ,3.59 ,3.66
1160 DATA 3.72 ,3.77 ,3.82 ,3.87 ,3.92 ,3.96 ,4.01 ,4.05 ,4.08 ,4.12
1170 DATA 4.16 ,4.19 ,4.23 ,4.26 ,4.29 ,4.33 ,4.36 ,4.39 ,4.42 ,4.45
1180 DATA 4.48 ,4.50 ,4.53 ,4.56 ,4.59 ,4.61 ,4.64 ,4.67 ,4.69 ,4.72
1190 DATA 4.75 ,4.77 ,4.80 ,4.82 ,4.85 ,4.87 ,4.90 ,4.92 ,4.95 ,4.97
1200 DATA 5.00 ,5.03 ,5.05 ,5.08 ,5.10 ,5.13 ,5.15 ,5.18 ,5.20 ,5.23
```

```
1210 DATA 5.25 ,5.28 ,5.31 ,5.33 ,5.36 ,5.39 ,5.41 ,5.44 ,5.47 ,5.50
1220 DATA 5.52 ,5.55 ,5.58 ,5.61 ,5.64 ,5.67 ,5.71 ,5.74 ,5.77 ,5.81
1230 DATA 5.85 ,5.88 ,5.92 ,5.95 ,5.99 ,6.04 ,6.08 ,6.13 ,6.18 ,6.23
1240 DATA 6.28 ,6.34 ,6.41 ,6.48 ,6.55 ,6.64 ,6.75 ,6.88 ,7.05 ,7.33
1250 NEXT I
1260 DIM TC( 84 ,3 )
1270 FOR I=16 TO 84
1280 FOR J=1 TO 3
1290 READ TC( I ,J ).
1300 DATA 1.33 ,8.115 ,.005 ,1.42 ,5.805 ,.006 ,1.51 ,4.194 ,.008
1310 DATA 1.60 ,3.061 ,.011 ,1.70 ,2.256 ,.015 ,1.79 ,1.680 ,.019
1320 DATA 1.88 ,1.263 ,.0.25 ,1.97 ,.959 ,.031 ,2.06 ,.736 ,.04
1330 DATA 2.15 ,.57 ,.05 ,2.23 ,.446 ,.2274 ,.11 ,2.58 ,.185 ,.131
1340 DATA 2.41 ,.282 ,.092 ,2.49 ,.2274 ,.11,2.58 ,.185 ,.131
1350 DATA 2.66 ,.152 ,.154 ,2.74 ,.127 ,.18 ,2.83 , .106 , .208
1360 DATA 2.91 , 09 , .238 ,2.98 , .077 ,.269 ,3.06 ,.067 ,.302
1370 DATA 3.14 , .058 , .336 ,3.21 ,.051 ,.37 ,3.28 ,.046 ,.405
1380 DATA 3.34 , .041 ,.439 ,3.41 ,.038 ,.471 ,3.47 ,.034 ,.503
1390 DATA 3.53 ,.032 ,.532 ,.3.58 ,.03 ,.558 ,3.62 ,.028 , .581
1400 DATA 3.66 , .027 , .601 ,3.7 , .026 , .616 ,3.72 ,.026 ,.627
1410 DATA 3.74 , .025 , .634 ,3.75 ,.025 ,.637 ,3.74 ,.025 , .634
1420 DATA 3.72 ,.026 ,.627 ,3.68 , .026 , .616 ,3.62 , .027 , .601
1430 DATA 3.54 , .028 , .581 ,3.42 , .03 , .558 ,3.27 , .032 , .532
1440 DATA 3.08 , .034 , .503, 2.83 , .038 , .471 ,2. 52 , .041 , .439
1450 DATA 2.13 , .046 , .405 ,1.64 , .051 , .37 ,1.03 , .058 , .336
1460 DATA .26 , .067 , .302 ,-.71 , .077 , .269 , -1.92 , .09 , .238
1470 DATA -3.46 , .106 , .208 , -5.41 , .127 , .18 ,-7.9 , .152 , .154
1480 DATA -11.1 , .185 , .131 , -15 . 23 , .227 , . 11 ,-20.6 , .282 , .092
1490 DATA -27.62 , .353 , .076 ,-36.89 , .446 , .062 ,-49 .2, .57,.05
1500 DATA -65.68 , . 736 , .04 , -87.93 , .959 , .031 ,-118.22 ,1.263 , .025
1510 DATA 159.8 ,1.68 , .019 ,-217.3,2.256 , . 015, -297 .7 ,3.06 , . O11
1520 DATA -410.9 , 4.194 , . 008 , -571 .9,5.805 , .006 , -802.8 ,8.115 , .005
1530 NEXT J : NEXT I
```

```
1540 DIM TA( 20 )
1550 FOR I=1 TO 20
1560 READ TA( I )
1570 DATA 3.84 ,5.99 ,7.81 ,9.49 ,11.07 ,12.59 ,14.07 ,15.51 , 16.92 , 18.31
1580 DATA 19.68 ,21.03 ,22.36,23.68 ,25.00 , 26.30 ,27.59 ,28.87 ,30.14 ,31.41
1590 NEXT I
1600 CLOSE #1
1610 RETURN
1620 REM PRINT TABLE
1630 IF I>1 THEN GOTO 1640
1640 PRINT "Dose " SPC( 3 ) " Ldos" SPC( 2 ) "#U" SPC( 2 ) "KI" SPC( 2 ) "%Kill SPC( 2 )
"Cr%" SPC( 2 ) "E Pr" SPC( 2 ) "Ex Pr" SPC( 2 ) "WK Pro " SPC( 2) "Weght" SPC( 2 ) "F pro"
1645 PRINT STRING$( 70 , 196)
1650 PRINT #2 , " Dose " SPC( 3 ) " Ldos " SPC( 2 ) "#U" SPC( 2 ) "KI" SPC( 2 ) "%Kill"
SPC( 2 ) "Cr% "" SPC( 2 ) "E Pr" SPC( 2 ) "Ex Pr" SPC( 2 ) "Wk Pro" SPC( 2) "Weght" SPC(2)
"F pro"
1655 PRINT#2 ,STRING$( 70 ,196 )
1680 FPR I = 1 TO D
1690  PRINT USING "### . ### "; DS( I ) ; : PRINT USING "#### . ### " ; DL( I ) ;
1691 PRINT USING "####" ; N( I ) ; : PRINT USING " ####" ;K( I ) ;
1692 PRINT USING " #### . ### ";P( I ) ; : PRINT USING " #### "; PC( I )
1693 PRINT USING "### . ## ";EP( I ) ; : PRINT USING " ### . ### ";EY( I )
1694 PRINT USING  "#### . ### ";WP( I ) ; :PRINT USING "#### . ## "; WT( I ) ;
1695 PRINT USING "### . ### ";FP( I )
1700  PRINT#2 , USING "### . ### " ;DS( I ) ; " PRINT#2 , USING "#### . ### "; DL( I ) ;
1701 PRINT#2 , USING "####" ;N( I ) ; : PRINT#2 , USING "####";K( I ) ;
1702 PRINT#2 , USING "#### . ### " ;P( I ) ; : PRINT#2 , USING "#### "; PC( I )
1703 PRINT#2 , USING "### . ## ";EP( I ) ; : PRINT#2 , USING "### . ### ";EY( I ) ;
1704 PRINT#2 , USING "#### . ### ";WP( I ) ; : PRINT#2, USING "#### . ## ";WT( I ) ;
1705 PRINT#2 , USING "### . ### : ;FP( I )
1710  NEXT
1715 PRINT#2 , STRING$( 70 , 196 )
1717 PRINT STRING$( 70, 196 )
```

```
1720 PRINT : PRINT#2,
1730 RETURN
1740 PRINT "ESTIMATE ";J%
1750 PRINT#2 , "ESTIMATE "; J%
1760 PRINT "Y = ";A;" + ";B;" x "
1770 PRINT#2 , "Y = ";A; " + " ; B ;" x "
1780 PRINT"'
1790 PRINT#2 ,
1800 RETURN
1810 PRINT "ERROR AT LINE "; ERL
1820 PRINT : INPUT "do you want graph ( y / n ) "; A$
1830 IF A$ = "y" THEN 1850
1840 IF A$ = "n" THEN STOP
1850 OPEN "o" ,#1 , "dat2 "
1860 FOR I = 1 TO D
1870 PRINT #1 , DL( I ) , FP( I )
1880 NEXT I
1890 CLOSE #1
1900 CHAIN " PROG2 "
```