ANTI-INFLAMMATORY AND ANTI-BACTERIAL ACTIVITY OF SOUTH AFRICAN *Erythrina* SPECIES

By

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requirements for the degree
of Masters in Science
in the School of Botany and Zoology,
University of Natal, Pietermaritzburg

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Nature hangs green curtains round
the edges so that we-shan't probe
into the secrets that are hidden
somewhere in the depths of space
beyond the range of sight
But if we will only turn the key
We will unlock the gold

Anon

PREFACE

The experimental work described in this dissertation was carried out in the Department of Botany, University of Natal, Pietermaritzburg, under the supervision of Professor J. van Staden and Doctor A.K. Jäger.

These studies represent work done by the author and have not otherwise been submitted in any form for any degree or diploma to any other University. Where use has been made of the work of others it is duly acknowledged in the text.

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Candice Claudia Natasha Pillay

We declare that the above statement is true

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J. van Staden - Supervisor

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A.K. Jäger - Co-supervisor

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Sincere thanks are due to many individuals who contributed to this project:

To my supervisor Professor J. van Staden for his guidance and support.

To my co-supervisor Doctor A.K. Jäger for her invaluable insight, guidance and encouragement.

To members of staff in the Department of Botany for technical advice and help.

To my parents for their unfailing love and encouragement. Throughout my studies they have been my pillar of strength.

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ABSTRACT

An investigation was undertaken to determine whether *Erythrina* species indigenous to South Africa contained the same type of compounds as *Erythrina* species not found in South Africa and to determine whether they displayed any anti-inflammatory and anti-bacterial activity.

Phytochemical analysis was conducted using thin layer chromatography. A great similarity was found in the leaf profiles of the species being studied. The leaf and bark extracts of *E. caffra* and *E. lysistemon* appear to have similar profiles when viewed under normal light and ultraviolet light, (254 and 366 nm). These two species have similar banding patterns when stained with fast blue reagent for flavonoids and potassium hydroxide reagent for coumarins. The five species that were tested appear to contain alkaloids, flavonoids, coumarins and triterpenes just like the species not found in South Africa from this genus.

Dried bark and leaves from *E. caffra, E. humeana, E. latissima, E. lysistemon* and *E. zeyheri* were screened for anti-inflammatory and anti-bacterial activity. Ethanol, ethyl acetate and water extracts were screened for both anti-inflammatory and anti-bacterial activity. The cyclooxygenase bioassay was used to test for anti-inflammatory activity. The ethanol and ethyl acetate extracts generally displayed activity while the water extracts displayed no activity for both the bark and the leaves. The bark generally displayed more cyclooxygenase inhibitory activity than the leaves. The bark of *E. caffra* and *E. lysistemon* displayed the highest cyclooxygenase inhibitory activity.

The disc diffussion bioassay was used to screen for anti-bacterial activity. Anti-bacterial activity was only detected in the water extracts of the leaves. The water extracts of the bark showed very little or no activity. The bark yielded more anti-bacterial activity than the leaves. Anti-bacterial activity was mainly displayed against Gram positive bacteria. The bark of *E. caffra* and *E. lysistemon* displayed the highest anti-bacterial activity.

On the basis of the screening results it was decided to use bioasssay guided fractionation in an attempt to isolate putative anti-inflammatory and anti-bacterial compounds. A hexane extract from the bark of *E. lysistemon* was prepared and purified using a range of chromatographic methods. Vacuum liquid chromatography, separation using a chromatotron, thin layer chromatography and high performance liquid chromatography were used to isolate anti-inflammatory compound(s). The isolation proved to be unsuccessful as the pure compound had no cyclooxygenase inhibitory activity. It was subsequently determined that the compounds were lost during the HPLC procedure.

An ethanolic extract of the bark of *E. lysistemon* was purified in an attempt to isolate an anti-bacterial compound(s). Vacuum liquid chromatography and separation using the chromatotron was used to purify the crude extract. The more sensitive microtitre bioassay was used to test for anti-bacterial activity against *S. aureus*. The isoflavone, Wighteone was isolated.

PUBLICATIONS FROM THIS THESIS

C.C.N. Pillay, A.K. Jäger and J. van Staden - Anti-inflammatory and Anti-bacterial activities of South African *Erythrina* Species. Submitted to the Journal of Ethnopramacology.

C.C.N. Pillay, A.K. Jäger and J van Staden - Erythrinin B, an anti-bacterial isoflavone from the bark of *Erythrina lysistemon.* In preparation, to be submitted soon.

CONFERENCES ATTENDED

Conference for Indigenous Plant Use Research 1998 :

Presented a poster titled - Screening Indigenous Erythrina species for Anti-inflammatory and Anti-bacterial Activity.

25th Annual South African Association of Botanists Conference :

Presented a paper titled - Biological Activity of South African *Erythrina* Species

2000 Years of Natural Products Conference in Amsterdam :

Presented a poster titled - Biological Activity of South African *Erythrina* Species

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ABBREVIATIONS

TLC - Thin layer chromatography

HPLC - High performance liquid chromatography

MIC - Minimum inhibitory activity

NMR - Nuclear magnetic resonance

UV - Ultraviolet

CHAPTER ONE
LITERATURE REVIEW

1.1 TRADITIONAL MEDICINE

For centuries there has been a conviction in the health profession and academia that traditional health systems and practices would fade away in modern and industrialized societies. Unfortunately, or maybe fortunately, traditional healing systems have persisted alongside the rapidly growing and changing medical establishment. As we enter the 21st century the major pharmaceutical companies and medical research groups are realising the enormous potential of indigenous herbs as sources of new drugs.

In South Africa the demand for indigenous medicines and services is considerable relative to the demand for Western health care services. The dire shortage of doctors in rural areas has forced inhabitants to consult with traditional healers, if not by choice, then certainly by necessity (VEALE, FURMAN & OLIVIER, 1992). It is estimated that 27 million consumers in South Africa utilize indigenous medicine and services. Households in South Africa are spending between 4% and 8% of their annual income on indigenous medicine and services (MANDER, 1998). From these statistics one cannot dispute the importance of the traditional healer in South Africa.

A. Traditional Healers

There are three types of traditional healers in South Africa (PANTANOWITZ,1994):

i. Sangoma (diviner) - They are spiritualists providing a link between the living and the dead. They use the divination of bones;

ii. Nyanga (herbalist) - They use plant extracts as remedies; and

iii. Mthakathi (sorcerer) - They practice "black magic". They use human tissue for "muthi". A popular ingredient is human testis. It is believed to increase the potency of the "muthi".

African traditional healers have an elaborate *materia medica*. They combine the work

of the clinician and the pharmacist as practised by Western medicinal concepts (IWU,1993). Traditional healers acquire their knowledge and skills from a family member or through apprenticeship to a renowned practitioner (NYAMWAYA, 1992). Official herbalists need to be registered. Registration is based on the hereditary chiefs in whose area the herbalist resides. The registration is covered by the KwaZulu/Natal Act on the code of the Zulu law; Act 6 of 1981. Registration of herbalists is, however, less effective now than it was before (CUNNINGHAM, 1988). Registration is seen as an attempt to control the practice of the herbalist. It is required to show that a herbalist has been trained by a professional herbalist and also to control the dispensing of herbal remedies. As much as registration could have a positive effect it does not eliminate the fact that herbal remedies sometimes have deleterious and harmful effects on patients.

B. Negative aspects of traditional medicine

Diagnosis of an illness by a traditional healer could be problematic. Medical doctors have the latest medical equipment and have been trained to use it so that they could make accurate diagnosis. Traditional healers generally do not have any medical equipment that they can use to make diagnosis. They usually listen to the symptoms that the patient describes and make inferences from their knowledge about a certain disease or illness. This is by no means an accurate diagnosis (PANTANOWITZ, 1994).

The dosage of the prescribed medicine could cause a serious problem (NJAU, 1991). Herbs growing in hot, dry areas have a different concentration of active ingredients from those growing in wet, cold areas. The age of a particular plant could also influence the concentration of active ingredients. The traditional healer needs to be aware of this and also how the environment could influence the concentration of active compounds (NYAMWAYA, 1992). However, without any scientific training or any other education how can a traditional healer possibly know this? Some herbs are also extremely toxic, an increase in dosage beyond a certain point can therefore be fatal. Certain herbs are toxic but traditional healers are usually not aware of the toxicity. In South Africa toxic species such as *Callilepis laureola* (impila) are being dispensed by traditional healers despite the fact that it is known to have caused 263 reported deaths, 44% of which were children under the age of 10 years (WAINWRIGHT, SCHONLAND & CANDY, 1977 in

CUNNINGHAM, 1988).

A number of indigenous procedures involve incisions, scarifications or excisions. The medical efficacy of these procedures are questionable (PANTANOWITZ, 1994). These procedures are off course not performed under complete sterile conditions making many of them very dangerous. Septic infections, trauma due to excessive bleeding and shock are some of the minor effects that these blood letting procedures could have. The more serious effects would be the transmission of diseases such as AIDS and in some cases death (NYAMWAYA, 1992). What is interesting is that most people who have these procedures performed on them and even the traditional healers who perform them are unaware of the risks.

The most dangerous practice by traditional healers is the claim that they can cure all illnesses and diseases, including AIDS. While it is possible that traditional medicine could have some sort of impact on some illnesses, it is not true that any practitioner whether it be Western or traditional can cure all illnesses and diseases (O'CONNOR, 1995). These wild claims usually lead to delayed referral for appropriate treatment, which could have detrimental consequences.

Even though there are a large number of negative aspects to traditional medicine there are however, also many positive aspects related to traditional medicine.

C. Positive aspects of traditional medicine

Traditional medicine provide inhabitants of rural areas with some form of health care where Western health care facilities are often lacking (BYE & DUTTON, 1991). In 1982 it was estimated that there is approximately one medical practitioner for every 17 500 people in South Africa (VEALE, FURMAN & OLIVIER,1992). With these statistics it is clear that traditional medicine should intervene and provide some sort of health care.

To a large number of people illness is not only a physical phenomenon. Social and spiritual factors are also expected to be involved in illness and disease in some way (O'CONNOR, 1995). For some people the spiritual and social factors need to be

addressed and dealt with before complete healing is achieved. In this case the traditional healer performs rituals and provides a psychological support system for the patient. This relieves people of their fears and restores their confidence to face life's challenges.

The remedies administered by traditional healers relieves the physical symptoms of illness. A number of herbal remedies do actually cure the ailment that they are used for. For example, Joy Veale, a lecturer at the University of the Witwatersrand screened an extract of *Clivia miniata* which is used by black South African women to induce labour. She proved that the *Clivia* behaved in a similar way as ergometrine, causing contractions of the uterine muscle (PANTANOWITZ, 1994).

D. Interaction between indigenous and Western forms of health care

There is generally very little interaction between indigenous and Western health care systems, if any. The interaction that does exist is usually negative. Western medicine is often considered superior to traditional remedies and the direct use of plants is pushed into the background (HOSTETTMANN & MARSTON, 1987). There exists a great amount of intolerance between the two parties for a number of reasons. The cosmopolitan health care workers are of the impression that traditional healers stop people from using the Western health care facilities and medicines available to them. Some traditional healers attempt to build bridges of communication by referring patients with illnesses that they cannot cure to Western health care facilities (NYAMWAYA, 1992). Western doctors almost always reprimands a patient that admits to having received traditional health care. Patients are therefore reticent to admit either using or administering traditional remedies (BYE & DUTTON, 1991).

Ethnobotanists need to throw the light of science on the herbalists art. They need to lay down a more pragmatic and scientific basis for this practice. If ethnobotanists can carry out research aimed at laying a firm foundation and a rational scientific basis for herbal medicine, then maybe cosmopolitan health care systems would have a more positive attitude towards traditional medicine. If the results of scientific research is readily available to herbalists through education, interaction and mutual exchange of ideas, then scientists will be making a very useful contribution to herbal medicine and health care

in general. The first step towards developing systems of shared knowledge, use and benefits is to draw together all information, this is what ethnobotany encompasses.

1.2 ETHNOBOTANY

Ethnobotany can be defined in a number of different ways, i.e. (GIVEN & HARRIS, 1994) :

i. The study of the relationships between people and plants and especially the utilization of plants by people;

ii. The study of the past and present interrelations of primitive or aboriginal human societies with the ambient vegetation; or

iii. The study and evaluation of the knowledge of all phases of plant life amongst primitive societies and the effect of the vegetal environment upon the life, customs, beliefs and history of the people of such societies.

Ethnobotany is a science which brings cultural awareness of natural resources and is applied specifically to tribal people.

A. People, plants and culture

The dependance of mankind on plants is as ancient as our evolutionary history. People have depended on plants to satisfy the needs of daily life; food, shelter, clothing, fuel and medicines (MCKENNA, 1992). The most important of these uses being food. The selection of some plants in the past has led to the development of major crops such as maize, rice, barley, wheat and potato. Some of these crops have become important staple foods for specific tribes thus becoming a facet of their culture (BALICK & COX, 1996).

The medicinal properties of plants cannot be overlooked especially in rural areas. Thousands of plants are used by tribal people for their medicinal properties, for example, in Samoa some tribes use the bark, roots and meristems of *Inocarpus fagifer* for appendicitis, internal distress and inflammation (COX, 1993). In South Africa the Xhosa people use *Hypoxis latifolia* for heart complaints, impotency and barrenness (SIMON &

LAMLA, 1991). The uses stated above are merely examples of traditional medicinal plants used by tribal people; it is by no means indicative of even the smallest proportion of plants used by tribal people for their medicinal properties. The number of plants used for their medicinal properties are endless. COX and BALICK (1994) stated that 265 000 flowering species grace the earth and less than half of one percent have been studied exhaustively for their chemical composition and medicinal value. Investigations of the medicinal properties of plants have revealed and are still yielding compounds which have applications worldwide.

B. Ethnobotany a multi-disciplinary science

Ethnobotany is truly a multi-disciplinary science that has come of age. It incorporates biology, plant science, chemistry, history, anthropology, culture and literature. It is dependant on the close collaboration with the above mentioned disciplines (GIVEN & HARRIS, 1994). Other essential collaborators who have also been involved in the ethnobotanical plight are the pharmacists, pharmacologists and ethnopharmacologists. Ethnobotany has also given rise to the discipline, ethnomedicine. In the case of ethnomedicine there is a need for the involvement of physicians in field work to assist with the diagnosis of symptoms treated by medicinal herbs (PRANCE, 1994). Collaboration can only be achieved through centres of excellence.

C. Ethical issues involved in ethnobotany

A number of ethical issues will always arise when conducting an ethnobotanical study. The most important of these issues being intellectual property rights. It is easy to assume that the absence of formal plant rights and patenting for plants means that anyone is free to obtain information and to use it to their advantage (GIVEN & HARRIS, 1994). Commercial enterprises must recognize the need to share benefits. The benefits need to be shared with the countries that are the source of genetic resources and more importantly with the indigenous people (MARTIN, 1995). Intellectual property rights allows only for the patenting of identified active plant compounds. It does not include the plant or indigenous information, so indigenous people cannot use it to assure that they are rewarded for their information. In developed countries indigenous people do have rights that provide a basis for trade secret protection. This allows pharmaceutical

organisations to develop and market products as long as the indigenous people are properly rewarded (BARTON, 1994). This raises the question; " What is an adequate reward and who decides on the reward?"

1.3 DRUG DISCOVERY

A. The role of plants in drug discovery

Even though the first chemical substance isolated from plants was benzoic acid in 1560, it was not until the 18th century that the search for useful drugs started (FARNSWORTH, 1984). A British physician, William Withering in 1785 found that the leaves of *Digitalis purpurea* could be used to treat dropsy (COX & BALICK, 1994). This led to the isolation of digoxin and digitoxin from this genus (COX, 1994). These compounds along with other cardiac glycosides isolated from the genus *Digitalis* are presently prescribed to thousands of cardiac patients annually. In 1804 morphine was isolated from the dried latex of *Papaver somnifermum L.* (opium) (FARNSWORTH, 1984). Many useful drugs from higher plants have since been discovered.

Plants seem to have served as models for drug development for three reasons (FARNSWORTH, 1994) :

i. Twenty five percent of all prescription drugs contain active compounds from higher plants;

ii. Biologically active compounds sometimes have poor pharmacological activity in humans and in this case these compounds are used as templates for synthetic modification and or manufacturing; and

iii. Secondary plant metabolites are useful in studying biological systems and disease processes.

There is presently a number of plant-derived drugs being used throughout the world. Table 1.3.1 shows some of these drugs.

Table 1.3.1 Plant-derived drugs currently being used worldwide (FARNSWORTH, 1984)

Acetyldigoxin	Ephedrine	Pseudoephedrine
Aescin	Hyoscyamine	Quinidine
Ajmalicine	Khellin	Quinine
Allantoin	Lanatoside	Rescinnamine
Atropine	Leurocristine	Reserpine
Bromelain	α-Lobeline	Scillarens A & B
Codeine	Morphine	Scopolamine
Colchecine	Narcotine	Sennosides A & B
Danthron	Ouabain	Sparteine
Deserpidine	Papain	Strychnine
Digtoxin	Papaverine	Tetrahydrocannabinol
Digoxin	Physostigmine	Theobromine
L-Dopa	Pilocarpine	Theophylline
Emetine	Protovertarines A & B	Tubocuranine
		Xanthotoxin

B. Ethnobotanical approach to drug discovery

The ethnobotanical approach to drug discovery is based on the assumption that indigenous uses of plants offers clues to the biological activities that those plants display. It is one of several methods that could be used to choose plants for pharmacological studies (COX & BALICK, 1994). The ethnobotanical approach has resulted in three types of drug discovery (COX, 1994) :

i. Unmodified plant products that have actually displayed clinical efficacy, e.g. digoxin;

ii. Unmodified plant products where its use was only remotely suggested by its indigenous use, e.g. vincristine; and

iii. Synthetic products based on natural products, e.g. aspirin.

Drug development from ethnobotanical leads is a long and ardous procedure but a number of drugs have been discovered in this manner. While incomplete Table 1.3.2

contains at least 50 such drugs.

Table1.3.2. Drugs discovered by ethnobotanical leads (COX, 1994)

Drug	Medical use	Plant source
Ajmaline	Heart arrhythmia	*Rauvolia* spp
Aspirin	Anti-inflammatory	*Filipendula ulmaria*
Atropine	Pupil dilator	*Atropa belladonna*
Benzoin	Oral disinfectant	*Styrax tonkinensis*
Caffeine	Stimulant	*Camellia sinensis*
Camphor	Rheumatic pain	*Cinnamomum camphora*
Cascara	Purgative	*Rhamus purshiana*
Cocaine	Ophthalmic anaesthetic	*Erythoxylum coca*
Codeine	Analgesic, antitussive	*Papaver somnifermum*
Colchicine	Gout	*Colchicium autumnale*
Demecolcine	Leukaemia, lymphomata	*C. autumnale*
Deserpidine	Anti-hypertensive	*Rauvolfia canescens*
Dicoumarol	Antithromobotic	*Melilotus officinalis*
Digoxin	Atrial fibrillation	*Digitalis purpurpea*
Digitoxin	Atrial fibrillation	*Digitalis prupurpea*
Emetine	Amoebic dysentery	*Psychotria ipecacuanha*
Ephedrine	Bronchodilator	*Ephedra sinica*
Eugenol	Toothache	*Syzygium aromaticum*
Gallotanins	Haemorrhoid suppository	*Hamamelis virginia*
Hyoscyamine	Anticholinergic	*Hyoscyamus niger*
Ipecac	Emetic	*Psychotria ipecacuanha*
Ipratropium	Bronchodilator	*Hyoscyamus niger*
Morphine	Analgesic	*Papaver somnifermum*
Noscapine	Antitussive	*Papaver somnifermum*
Papain	Attenuator of mucus	*Carica papaya*
Papaverine	Antispasmodic	*Papaver somnifermum*
Physostigmine	Glaucoma	*Physostigma venenosum*
Picrotoxin	Barbiturate antidote	*Anamirta cocculus*
Pilocarpine	Glaucoma	*Pilocarpus jaborandi*
Podophyllotoxin	Condyloma acuminatum	*Podophyllum peltatum*
Proscillaridin	Cardiac malfunction	*Drimia maritima*
Protoveratine	Anti-hypertensive	*Veratrum album*
Pseudoephedrine	Rhinitis	*E. sinica*
Psoralen	Vitiligo	*Psoralea corylifolia*
Quinine	Malaria prophylaxis	*Cinchona pubescens*
Quinidine	Cardiac arrhythmia	*Cinchona pubescens*
Rescinnamine	Anti-hypertensive	*R. serpentia*
Reserpine	Anti-hypertensive	*R. serpentia*
Sennoside A, B	Laxative	*Cassia angustifolia*
Scopalamine	Motion sickness	*Datura stramonium*
Sigmasterol	Steroidal precursor	*Physostigma venenosum*
Strophanthin	Congestive heart failure	*Stratophanthus gratus*
Tubocurarine	Muscle relaxant	*Chondrodendron tomentosum*
Teniposide	Bladder neoplasms	*Podophullum peltatum*
Tetrahydrocanabinol	Anti-emetic	*Cannabis sativa*
Theophylline	Diuretic, anti-asthmatic	*Camellia sinensis*
Toxiferine	Relaxant in surgery	*Strychnos guianensis*
Vinblastine	Hodgkin's disease	*Catharanthus roseus*
Vincristine	Paediatric leukaemia	*Catharanthus roseus*
Xanthotoxin	Vitiligo	*Ammi majus*

i. Strengths of the ethnobotanical approach :

The ethnobotanical approach is targeted more towards a specific plant species, genus or family (COX & BALICK, 1994). A researcher would find that a specific plant is used for a specific ailment. He would therefore screen plants for biological activity from the same genus or family. It is also targeted towards specific biological activities. If for example a researcher is looking for plants that display biological against HIV then he/she would screen plants that have inhibitory activity against viruses.

The ethnobotanical approach also allows for indigenous knowledge to be passed on, and to be shared for the benefit of all people.

ii. Limitations of the ethnobotanical approach :

Very few of the compounds that exhibit biological activity in laboratory tests will become new drugs. Some will be less potent than existing ones and others will prove to be toxic for human use (COX & BALICK, 1994). Most of the compounds isolated from plants will never make it to the drug development process let alone the market.

Drug discovery using the ethnobotanical approach is a time consuming process. It takes at least 20 years from the beginning to the final product (BYE & DUTTON, 1991). Needless to say it requires a great deal of money and it is always difficult to find organizations willing to invest money in such a project.

Traditional healers are very reluctant to share their knowledge. The curative art is kept with sanctity and secrecy. It is believed that the herbal medicines will lose their potency if revealed to other people (ADDAE-MENSAH, 1991). It is one of the greatest tragedies of the twentieth century when knowledge is not shared and recorded for the benefit of all people.

C. A plant - based drug development program

The process of developing drugs from natural products is a very long, complex and expensive process (CRAGG, BOYD, CHRISTINI, MAYS, MAZAN & SAUSVILLE, 1996). Figure1.3.1 illustrates the sequential process

CHAPTER ONE
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Even though there are a large number of negative aspects to traditional medicine there are however, also many positive aspects related to traditional medicine.

C. Positive aspects of traditional medicine

Traditional medicine provide inhabitants of rural areas with some form of health care where Western health care facilities are often lacking (BYE & DUTTON, 1991). In 1982 it was estimated that there is approximately one medical practitioner for every 17 500 people in South Africa (VEALE, FURMAN & OLIVIER,1992). With these statistics it is clear that traditional medicine should intervene and provide some sort of health care.

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D. Interaction between indigenous and Western forms of health care

There is generally very little interaction between indigenous and Western health care systems, if any. The interaction that does exist is usually negative. Western medicine is often considered superior to traditional remedies and the direct use of plants is pushed into the background (HOSTETTMANN & MARSTON, 1987). There exists a great amount of intolerance between the two parties for a number of reasons. The cosmopolitan health care workers are of the impression that traditional healers stop people from using the Western health care facilities and medicines available to them. Some traditional healers attempt to build bridges of communication by referring patients with illnesses that they cannot cure to Western health care facilities (NYAMWAYA, 1992). Western doctors almost always reprimands a patient that admits to having received traditional health care. Patients are therefore reticent to admit either using or administering traditional remedies (BYE & DUTTON, 1991).

Ethnobotanists need to throw the light of science on the herbalists art. They need to lay down a more pragmatic and scientific basis for this practice. If ethnobotanists can carry out research aimed at laying a firm foundation and a rational scientific basis for herbal medicine, then maybe cosmopolitan health care systems would have a more positive attitude towards traditional medicine. If the results of scientific research is readily available to herbalists through education, interaction and mutual exchange of ideas, then scientists will be making a very useful contribution to herbal medicine and health care

in general. The first step towards developing systems of shared knowledge, use and benefits is to draw together all information, this is what ethnobotany encompasses.

1.2 ETHNOBOTANY

Ethnobotany can be defined in a number of different ways, i.e. (GIVEN & HARRIS, 1994) :

i. The study of the relationships between people and plants and especially the utilization of plants by people;

ii. The study of the past and present interrelations of primitive or aboriginal human societies with the ambient vegetation; or

iii. The study and evaluation of the knowledge of all phases of plant life amongst primitive societies and the effect of the vegetal environment upon the life, customs, beliefs and history of the people of such societies.

Ethnobotany is a science which brings cultural awareness of natural resources and is applied specifically to tribal people.

A. People, plants and culture

The dependance of mankind on plants is as ancient as our evolutionary history. People have depended on plants to satisfy the needs of daily life; food, shelter, clothing, fuel and medicines (MCKENNA, 1992). The most important of these uses being food. The selection of some plants in the past has led to the development of major crops such as maize, rice, barley, wheat and potato. Some of these crops have become important staple foods for specific tribes thus becoming a facet of their culture (BALICK & COX, 1996).

The medicinal properties of plants cannot be overlooked especially in rural areas. Thousands of plants are used by tribal people for their medicinal properties, for example, in Samoa some tribes use the bark, roots and meristems of *Inocarpus fagifer* for appendicitis, internal distress and inflammation (COX, 1993). In South Africa the Xhosa people use *Hypoxis latifolia* for heart complaints, impotency and barrenness (SIMON &

LAMLA, 1991). The uses stated above are merely examples of traditional medicinal plants used by tribal people; it is by no means indicative of even the smallest proportion of plants used by tribal people for their medicinal properties. The number of plants used for their medicinal properties are endless. COX and BALICK (1994) stated that 265 000 flowering species grace the earth and less than half of one percent have been studied exhaustively for their chemical composition and medicinal value. Investigations of the medicinal properties of plants have revealed and are still yielding compounds which have applications worldwide.

B. Ethnobotany a multi-disciplinary science

Ethnobotany is truly a multi-disciplinary science that has come of age. It incorporates biology, plant science, chemistry, history, anthropology, culture and literature. It is dependant on the close collaboration with the above mentioned disciplines (GIVEN & HARRIS, 1994). Other essential collaborators who have also been involved in the ethnobotanical plight are the pharmacists, pharmacologists and ethnopharmacologists. Ethnobotany has also given rise to the discipline, ethnomedicine. In the case of ethnomedicine there is a need for the involvement of physicians in field work to assist with the diagnosis of symptoms treated by medicinal herbs (PRANCE, 1994). Collaboration can only be achieved through centres of excellence.

C. Ethical issues involved in ethnobotany

A number of ethical issues will always arise when conducting an ethnobotanical study. The most important of these issues being intellectual property rights. It is easy to assume that the absence of formal plant rights and patenting for plants means that anyone is free to obtain information and to use it to their advantage (GIVEN & HARRIS, 1994). Commercial enterprises must recognize the need to share benefits. The benefits need to be shared with the countries that are the source of genetic resources and more importantly with the indigenous people (MARTIN, 1995). Intellectual property rights allows only for the patenting of identified active plant compounds. It does not include the plant or indigenous information, so indigenous people cannot use it to assure that they are rewarded for their information. In developed countries indigenous people do have rights that provide a basis for trade secret protection. This allows pharmaceutical

organisations to develop and market products as long as the indigenous people are properly rewarded (BARTON, 1994). This raises the question; " What is an adequate reward and who decides on the reward?"

1.3 DRUG DISCOVERY

A. The role of plants in drug discovery

Even though the first chemical substance isolated from plants was benzoic acid in 1560, it was not until the 18th century that the search for useful drugs started (FARNSWORTH, 1984). A British physician, William Withering in 1785 found that the leaves of *Digitalis purpurea* could be used to treat dropsy (COX & BALICK, 1994). This led to the isolation of digoxin and digitoxin from this genus (COX, 1994). These compounds along with other cardiac glycosides isolated from the genus *Digitalis* are presently prescribed to thousands of cardiac patients annually. In 1804 morphine was isolated from the dried latex of *Papaver somnifermum L.* (opium) (FARNSWORTH, 1984). Many useful drugs from higher plants have since been discovered.

Plants seem to have served as models for drug development for three reasons (FARNSWORTH, 1994) :

i. Twenty five percent of all prescription drugs contain active compounds from higher plants;

ii. Biologically active compounds sometimes have poor pharmacological activity in humans and in this case these compounds are used as templates for synthetic modification and or manufacturing; and

iii. Secondary plant metabolites are useful in studying biological systems and disease processes.

There is presently a number of plant-derived drugs being used throughout the world. Table 1.3.1 shows some of these drugs.

Table 1.3.1 Plant-derived drugs currently being used worldwide (FARNSWORTH, 1984)

Acetyldigoxin	Ephedrine	Pseudoephedrine
Aescin	Hyoscyamine	Quinidine
Ajmalicine	Khellin	Quinine
Allantoin	Lanatoside	Rescinnamine
Atropine	Leurocristine	Reserpine
Bromelain	α-Lobeline	Scillarens A & B
Codeine	Morphine	Scopolamine
Colchecine	Narcotine	Sennosides A & B
Danthron	Ouabain	Sparteine
Deserpidine	Papain	Strychnine
Digtoxin	Papaverine	Tetrahydrocannabinol
Digoxin	Physostigmine	Theobromine
L-Dopa	Pilocarpine	Theophylline
Emetine	Protovertarines A & B	Tubocuranine
		Xanthotoxin

B. Ethnobotanical approach to drug discovery

The ethnobotanical approach to drug discovery is based on the assumption that indigenous uses of plants offers clues to the biological activities that those plants display. It is one of several methods that could be used to choose plants for pharmacological studies (COX & BALICK, 1994). The ethnobotanical approach has resulted in three types of drug discovery (COX, 1994) :

i. Unmodified plant products that have actually displayed clinical efficacy, e.g. digoxin;

ii. Unmodified plant products where its use was only remotely suggested by its indigenous use, e.g. vincristine; and

iii. Synthetic products based on natural products, e.g. aspirin.

Drug development from ethnobotanical leads is a long and ardous procedure but a number of drugs have been discovered in this manner. While incomplete Table 1.3.2

contains at least 50 such drugs.

Table1.3.2. Drugs discovered by ethnobotanical leads (COX, 1994)

Drug	Medical use	Plant source
Ajmaline	Heart arrhythmia	*Rauvolia* spp
Aspirin	Anti-inflammatory	*Filipendula ulmaria*
Atropine	Pupil dilator	*Atropa belladonna*
Benzoin	Oral disinfectant	*Styrax tonkinensis*
Caffeine	Stimulant	*Camellia sinensis*
Camphor	Rheumatic pain	*Cinnamomum camphora*
Cascara	Purgative	*Rhamus purshiana*
Cocaine	Ophthalmic anaesthetic	*Erythoxylum coca*
Codeine	Analgesic, antitussive	*Papaver somnifermum*
Colchicine	Gout	*Colchicium autumnale*
Demecolcine	Leukaemia, lymphomata	*C. autumnale*
Deserpidine	Anti-hypertensive	*Rauvolfia canescens*
Dicoumarol	Antithromobotic	*Melilotus officinalis*
Digoxin	Atrial fibrillation	*Digitalis purpurpea*
Digitoxin	Atrial fibrillation	*Digitalis prupurpea*
Emetine	Amoebic dysentery	*Psychotria ipecacuanha*
Ephedrine	Bronchodilator	*Ephedra sinica*
Eugenol	Toothache	*Syzygium aromaticum*
Gallotanins	Haemorrhoid suppository	*Hamamelis virginia*
Hyoscyamine	Anticholinergic	*Hyoscyamus niger*
Ipecac	Emetic	*Psychotria ipecacuanha*
Ipratropium	Bronchodilator	*Hyoscyamus niger*
Morphine	Analgesic	*Papaver somnifermum*
Noscapine	Antitussive	*Papaver somnifermum*
Papain	Attenuator of mucus	*Carica papaya*
Papaverine	Antispasmodic	*Papaver somnifermum*
Physostigmine	Glaucoma	*Physostigma venenosum*
Picrotoxin	Barbiturate antidote	*Anamirta cocculus*
Pilocarpine	Glaucoma	*Pilocarpus jaborandi*
Podophyllotoxin	Condyloma acuminatum	*Podophyllum peltatum*
Proscillaridin	Cardiac malfunction	*Drimia maritima*
Protoveratine	Anti-hypertensive	*Veratrum album*
Pseudoephedrine	Rhinitis	*E. sinica*
Psoralen	Vitiligo	*Psoralea corylifolia*
Quinine	Malaria prophylaxis	*Cinchona pubescens*
Quinidine	Cardiac arrhythmia	*Cinchona pubescens*
Rescinnamine	Anti-hypertensive	*R. serpentia*
Reserpine	Anti-hypertensive	*R. serpentia*
Sennoside A, B	Laxative	*Cassia angustifolia*
Scopalamine	Motion sickness	*Datura stramonium*
Sigmasterol	Steroidal precursor	*Physostigma venenosum*
Strophanthin	Congestive heart failure	*Stratophanthus gratus*
Tubocurarine	Muscle relaxant	*Chondrodendron tomentosum*
Teniposide	Bladder neoplasms	*Podophullum peltatum*
Tetrahydrocanabinol	Anti-emetic	*Cannabis sativa*
Theophylline	Diuretic, anti-asthmatic	*Camellia sinensis*
Toxiferine	Relaxant in surgery	*Strychnos guianensis*
Vinblastine	Hodgkin's disease	*Catharanthus roseus*
Vincristine	Paediatric leukaemia	*Catharanthus roseus*
Xanthotoxin	Vitiligo	*Ammi majus*

i. Strengths of the ethnobotanical approach :

The ethnobotanical approach is targeted more towards a specific plant species, genus or family (COX & BALICK, 1994). A researcher would find that a specific plant is used for a specific ailment. He would therefore screen plants for biological activity from the same genus or family. It is also targeted towards specific biological activities. If for example a researcher is looking for plants that display biological against HIV then he/she would screen plants that have inhibitory activity against viruses.

The ethnobotanical approach also allows for indigenous knowledge to be passed on, and to be shared for the benefit of all people.

ii. Limitations of the ethnobotanical approach :

Very few of the compounds that exhibit biological activity in laboratory tests will become new drugs. Some will be less potent than existing ones and others will prove to be toxic for human use (COX & BALICK, 1994). Most of the compounds isolated from plants will never make it to the drug development process let alone the market.

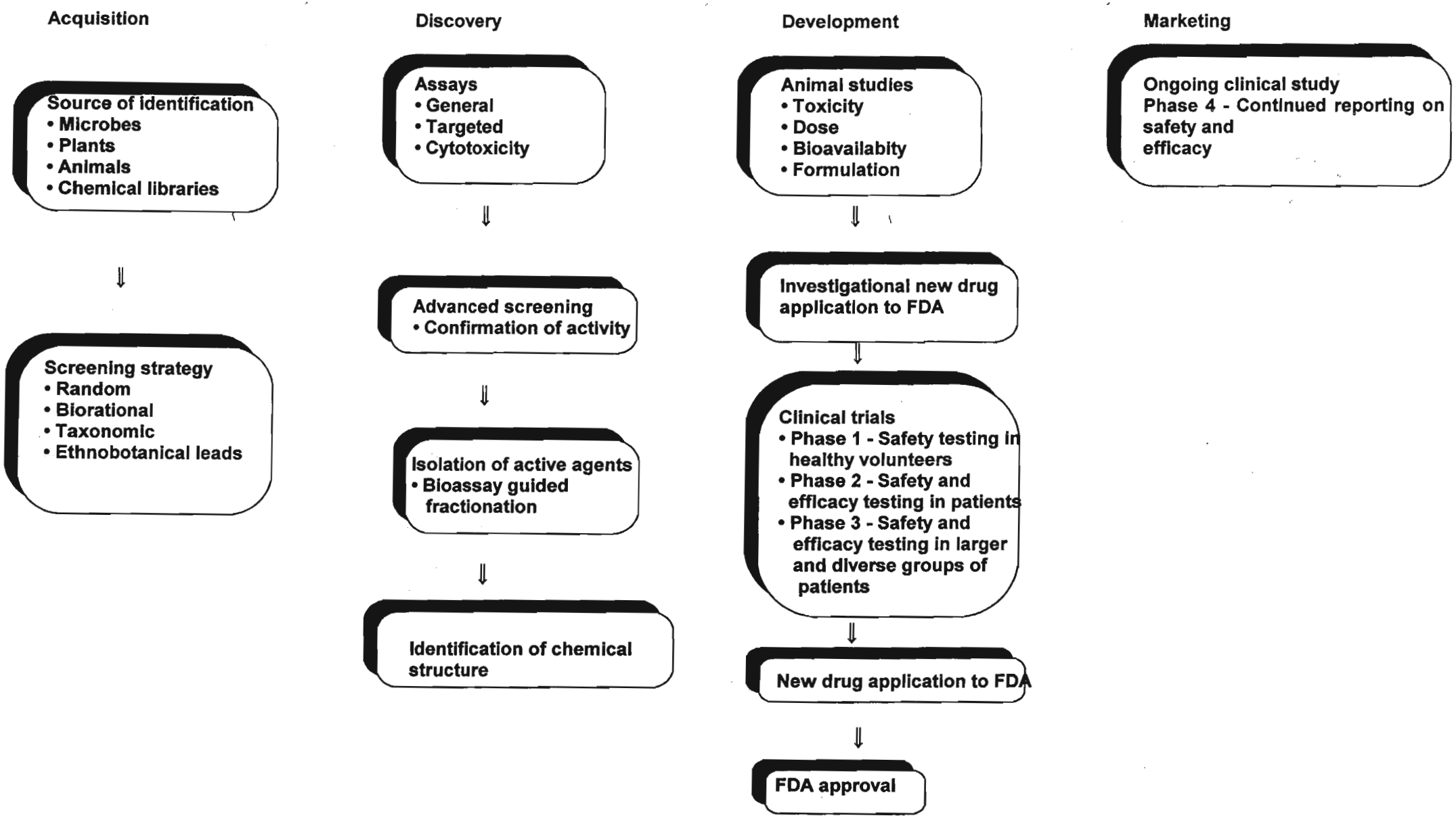
Drug discovery using the ethnobotanical approach is a time consuming process. It takes at least 20 years from the beginning to the final product (BYE & DUTTON, 1991). Needless to say it requires a great deal of money and it is always difficult to find organizations willing to invest money in such a project.

Traditional healers are very reluctant to share their knowledge. The curative art is kept with sanctity and secrecy. It is believed that the herbal medicines will lose their potency if revealed to other people (ADDAE-MENSAH, 1991). It is one of the greatest tragedies of the twentieth century when knowledge is not shared and recorded for the benefit of all people.

C. A plant - based drug development program

The process of developing drugs from natural products is a very long, complex and expensive process (CRAGG, BOYD, CHRISTINI, MAYS, MAZAN & SAUSVILLE, 1996). Figure1.3.1 illustrates the sequential process

Figure 1.3.1 A plant-based drug development program


Acquisition
Source of identification
• Microbes
• Plants
• Animals
• Chemical libraries
Screening strategy
• Random
• Biorational
• Taxonomic
• Ethnobotanical leads
Discovery
Assays
• General
• Targeted
• Cytotoxicity
Advanced screening
• Confirmation of activity
Isolation of active agents
• Bioassay guided fractionation
Identification of chemical structure
Development
Animal studies
• Toxicity
• Dose
• Bioavailabity
• Formulation
Investigational new drug application to FDA
Clinical trials
• Phase 1 - Safety testing in healthy volunteers
• Phase 2 - Safety and efficacy testing in patients
• Phase 3 - Safety and efficacy testing in larger and diverse groups of patients
New drug application to FDA
FDA approval
Marketing
Ongoing clinical study
Phase 4 - Continued reporting on safety and efficacy

CHAPTER TWO

Erythrina

Erythrina, a member of the family Fabaceae, often arborescent, grows in tropical and subtropical regions of the world. These trees are frequently referred to as coral trees because of their red seeds and flowers. Actually the name *Erythrina* is derived from the Greek word *erythros*, meaning red (VAN RENSBURG, 1982). There are approximately 120 species in existence, six of which are indigenous to South Africa. Of the six South African species four can be considered trees and two small shrubs.

2.1 SOUTH AFRICAN *Erythrina* SPECIES

A. *Erythrina acanthocarpa*

Common name: Tambookie thorn

Erythrina acanthocarpa grows naturally in the Queenstown district of the Eastern Cape Province (HENNESSY, 1972).

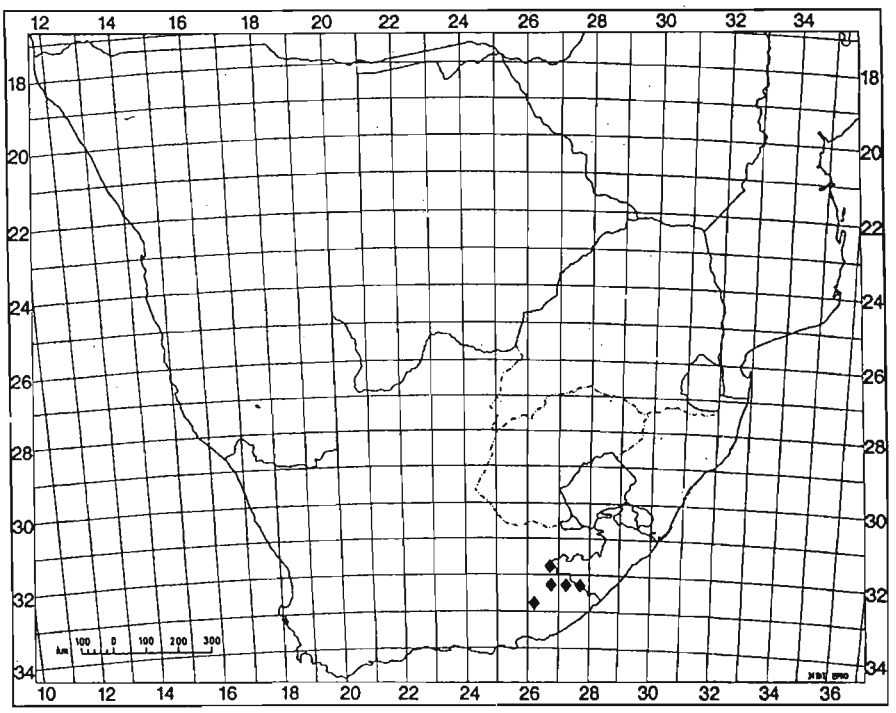


Figure 2.1.1 Distribution of *E. acanthocarpa* (HENNESSY,1991)

Figure 2.1.2 ***Erythrina acanthocarpa***

This shrub is usually 1 to 3 m tall and has large, tuberous roots. It does not die back completely in winter. It sheds all its leaves in winter but the branches live on until they bud again in spring. The leaves are small and the apical leaflet and the lateral leaflets are as long as they are broad. The leaves are blue-green in colour and prickles are found on the lower rib. This shrub flowers from late September. It produces tightly packed large spikes of flowers. These spikes can be 170mm long and 150mm wide (VAN RENSBURG, 1982). The standard petals are vermilion-red, tipped with yellow or

yellow suffused with green. This colour combination does not occur in any other of our indigenous *Erythrina's*. Its fruits are distinctive, being heavily armed with prickles. The seeds are larger than those of our other species and are brown not red. The fruit itself is brown, not blackish as it is for the other indigenous species. Propagation is mainly by seed, though cuttings sometimes set successfully (HENNESSY, 1972).

B. *Erythrina caffra*

Common names: Lucky bean tree, Cape kaffirboom, um Sinsi (Zulu)

Erythrina caffra is found in the Eastern Cape and southern KwaZulu Natal. It occurs as a forest tree especially along river banks (VAN RENSBURG, 1982).

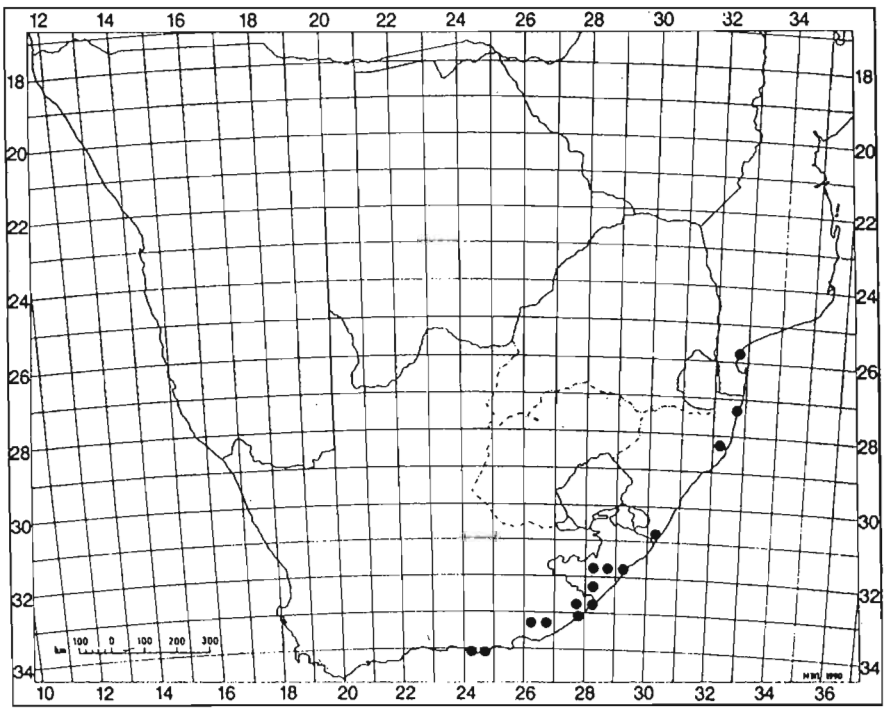


Figure 2.1.3 Distribution of *E. caffra* (HENNESSY, 1991)

Figure 2.1.4 ***Erythrina caffra***

These deciduous trees attain a height of 18m. The bark is grey green to light fawn in colour, with longitudinal fissures. Thorns are found on the trunk and the branches. Leaves are trifolate with an apical leaflet slightly larger than the lateral leaves. Both the apical and lateral leaflets are ovate to elliptical (PALGRAVE, 1977). These trees are winter to early spring flowering. The standard petal is usually a brilliant vermillion red, but there are terracotta, orange and cream coloured varieties too. The stamens of the flowers are exposed which is what distinguishes it from *E. lysistemon* (VAN

RENSBURG, 1982). The fruit and seeds of *E. caffra* and *E. lysistemon* are indistinguishable. The clusters of sinuous fruit in the infructescence present an interesting, Gorgonian appearance in contrast to the poised elegance of the inflorescences (HENNESSY, 1972).

E. caffra is easily propagated by means of cuttings, truncheons and seeds. It makes a fine ornamental tree but should not be planted too close to buildings because of its large root system. The trees are fast growing and make good shade trees in summer. They are however not frost tolerant (PALGRAVE, 1977).

C. *Erythrina humeana*

Common names: Dwarf lucky bean tree, Dwarf kaffirboom, umSinsana (Zulu)

This is a typically coastal species occurring especially along the Northern KwaZulu Natal coast. It is also found in the Eastern Cape, Mpumalanga, Swaziland and Mozambique (HENNESSY, 1972).

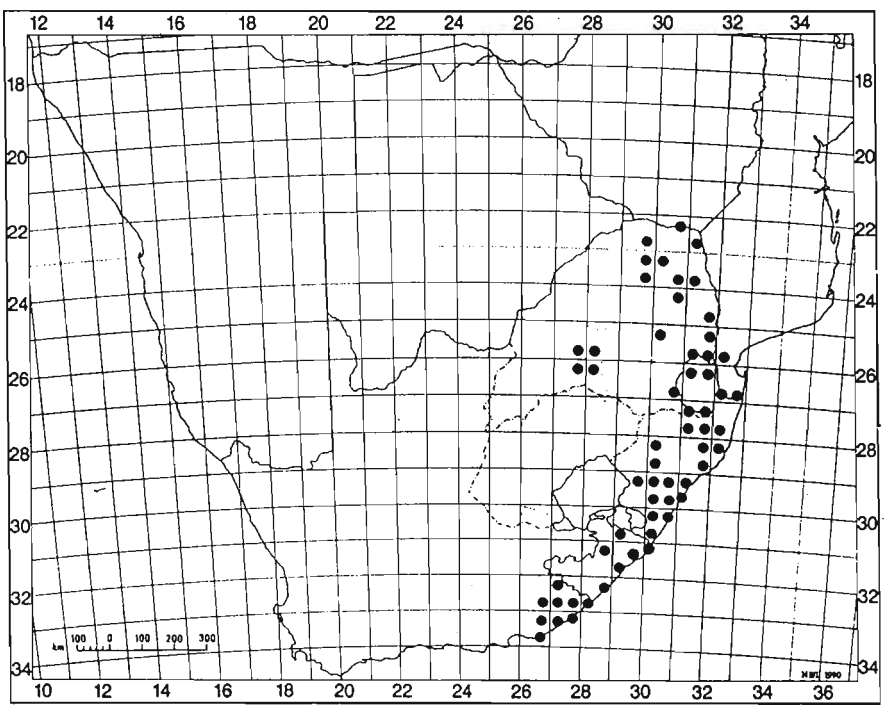


Figure 2.1.5 Distribution of *E. humeana* (HENNESSY, 1991)

Figure 2.1.6 ***Erythrina humeana***

These plants are slender graceful shrubs which attain a height of up to 3 m. They grow on coastal dunes, rocky outcrops and mountain slopes (VAN RENSBURG, 1982). The bark is grey and smooth with many thorns. Their shiny, dark green leaves sometimes look like that of *E. lysistemon.* Young leaves have tiny hairs but these disappear later. Main veins and stalks have prickles on them (PALGRAVE, 1977). These shrubs are summer to autumn flowering, the inflorescences appearing together with the leaves. The standard petals are deep scarlet in colour, becoming crimson with age; even the calices

calices are red (VAN RENSBURG, 1982). The fruit is very similar in appearance to those of both *E. caffra* and *E. lysistemon*, albeit a little smaller. The seeds of all three species are alike in appearance. Plants grow easily from seed but only rarely from cuttings which must be taken from old wood (HENNESSY,1972).

D. *Erythrina latissima*

Common names: Broad-leaved lucky bean tree, Cork tree, Broad-leaved coral tree, um Gqwabagqwaba (Zulu)

This coral tree is very different from the other coral trees; its leaves and flowers are much larger than that of the other trees. This tree is probably not as well known as the other coral trees but is lovely in its own right. The broad-leaved coral tree it is found in the Eastern Cape, KwaZulu Natal, Mpumalanga, Gauteng, Swaziland and the Mozambique highlands (VAN WYK, 1972).

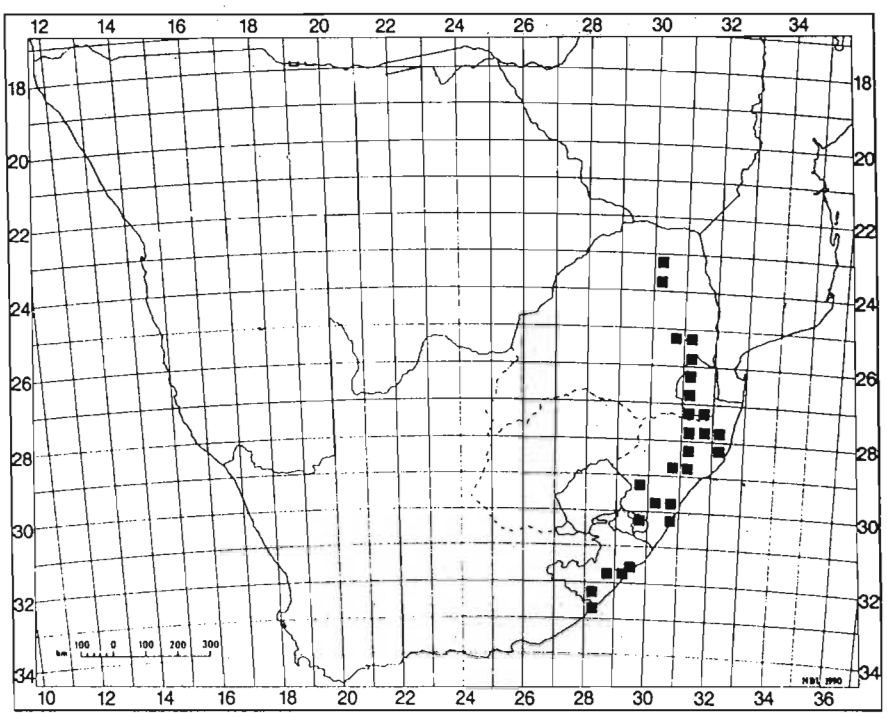


Figure 2.1.7 Distribution of *E. latissima* (HENNESSY, 1991)

Figure 2.1.8 ***Erythrina latissima***

The trees are not tall, attaining a height of 5 to 8 m at maturity. They are conspicuous for their thick grey corky bark and their large grey-green chartaceous leaves. The flowers are borne during the winter months and the early spring when the trees are virtually bare of leaves (PALGRAVE, 1977). The inflorescences are a magnificent deep scarlet colour and the calices are light pink and woolly. The calices are divided into slender lobes. As the flowers wither their colour changes; the petals shading to crimson

(HENNESSY, 1972). The fruit is a large woody legume, constricted between the seeds. It is brownish-black in colour when mature. It opens to reveal a creamy-beige interior. The seeds, like those of all legumes, are attached alternately near the upper mid-line of right and left halves of the pod. They have a hard, shiny red testa marked with a black scar, the hilum scar where they were attached to the pod (VAN RENSBURG, 1982).

Erythrina latissima can be propagated from large truncheons and can be grown from seed. It is able to withstand a limited amount of frost. Unfortunately, this species is nowadays far less common than it was because of man's injudicious use of fire as a quick and easy means to provide sprig grazing for livestock (HENNESSY, 1972)

E. *Erythrina lysistemon*

Common names: Lucky bean tree, Kaffirboom, umSinsi (Zulu)

It is because of its wide distribution and spectacular appearance during its flowering season, the best known of our erythrina's. It occurs in both low and high rainfall areas in the Eastern Cape, KwaZulu Natal, Gauteng, Swaziland, Botswana and Zambia (VAN WYK, 1972).

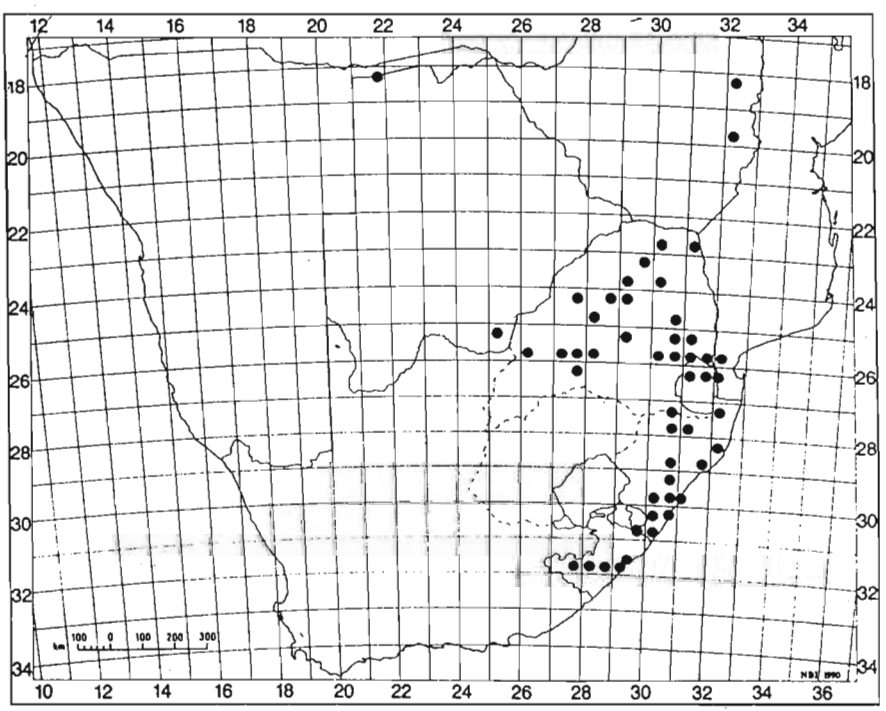


Figure 2.1.9 Distribution of *E. lysistemon* (HENNESSY, 1991)

Figure 2.1.10 ***Erythrina lysistemon***

The taller forms may attain a height of about 10 m, but most trees are only about 6m tall. The bark is rough, rather dark grey to grey-brown in colour. There are numerous purplish prickles on the branches. The leaves are trifolate with long stalks and are arranged spirally near the tips of branchlets (VAN RENSBURG, 1982). It flowers prolifically during winter and early spring. When the tree is in flower it is normally leafless. The inflorescences have a more restrained elegance than those of *E. caffra*. The bright scarlet flowers are arranged in short, closely-packed conical spikes (VAN

WYK, 1993). Pale pink and white variations of the flowers have been seen. The standard is never orange coloured. It is very easily propagated from cuttings, truncheons or seeds (HENNESSY, 1972).

F. *Erythrina zeyheri*

Common names: Plough breaker, Break harrow, Ploegbreker, umSinsana (Zulu)

It occurs in the midlands of KwaZulu Natal, the Free State, Lesotho and Gauteng (VAN RENSBURG, 1982).

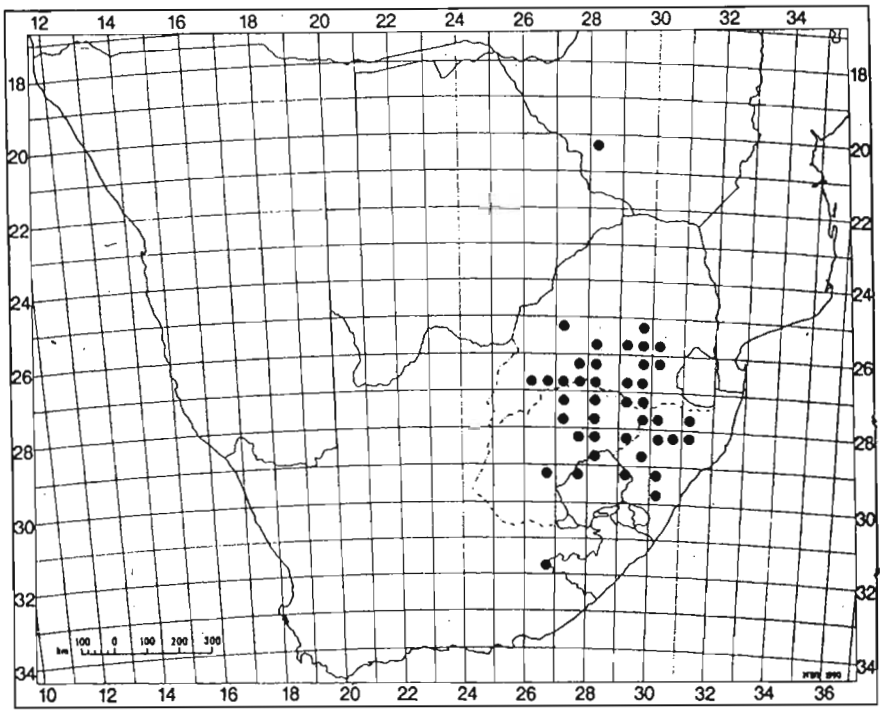


Figure 2.1.12 Distribution of *E. zeyheri* (HENNESSY, 1991)

Figure 2.1.12 ***Erythrina zeyheri***

The plough breaker is not a tree but a dwarf shrub. This shrub has a large underground stem and tuberous roots (hence its common name). The parts of this plant that are above the ground dies back completely during winter and sprouts again in spring. Large, trifolate leaves are found on this plant. The leaves are bright green in colour and has minute hairs. The leaf stalks are armed with prickles on both sides (HENNESSY,1972). These plants are spring flowering. The inflorescences are borne on long, longitudinally ridged peduncles, which are themselves often red in colour. The standards are bright scarlet and are shorter and blunter than those of other species. Because of the

acaulescent habit of this species, the only satisfactory means of propagation is from seeds (VAN RENSBURG, 1982).

2.2 CHEMICAL IMPORTANCE OF THE GENUS *Erythrina*

Species from this genus have since the early 1900's become the subject of extensive chemical research. Since the advent of these studies the genus *Erythrina* has become very popular for its alkaloids. This greatly stimulated chemical studies. Researchers have during the process of alkaloidal isolations stumbled on other types of compounds popular to this genus. These compounds are listed in the Table 2.2.1.

Table 2.2.1 Compounds isolated from *Erythrina* species

Erythrina	Geographical distribution	Compounds isolated	References
E. abyssinica	Angola, Burundi, Ethiopia, Kenya, Malawi, Mozambique, Rwanda, Sudan, Tanzania, Uganda, Zaire, Zambia, Zimbabwe & Mauritius	**Flavonoids :**	
		Abyssinone I, II, III, IV, V & VI, Callistephin, Cristacarpin, Cyanidin 3-sophoroside, Erythrabyssin II Pelargonidin 3-sophoroside Phasaeollidin & Phaseollin	SCOGIN, (1991)
		Abyssinin I, II & III, Sigmoidin A, B, C & F & Sigmoidin B 4'-(methyl ether)	ICHIMARU *et al.*, (1996)
		Amino acids & peptides :	
		Hypaphorine	FOLKERS, *et al.*, (1940)
		Alkaloids :	
		Erysodine	PRELOG, *et al.*, (1973)
		Erysopine	FOLKERS, *et al.*, (1940)
		Erysotrine, Erythratidine, 11-Methoxyerysovine & 11-Oxoerysodine	GAMES, *et al.*, (1974)
		Erysovine & Erythratine	BARAKAT, *et.al.*, (1977)
		Erythraline, Erythratine, Erythrastimine, Isoboldine, Orientaline & Glucoerysodine	BARTON, *et al.*, (1973)
E. acanthocarpa	Australia & South Africa	**Flavonoids :**	
		Callistephin & Pelargonidin	SCOGIN, (1991)
		Amino acids & peptides :	
		Hypaphorine (*S*)-form	FOLKERS *et al.*, (1941)

Erythrina	Geographical distribution	Compounds isolated	References
		Alkaloids :	
		Erysodine, Erysoline, Erysopine & Erysovine	HARGREAVES *et al.*, (1974)
E. americana	Mexico	**Aliphatic natural products :**	
		Hexadecanoic acid, Octadecanoic acid & Tetradecanoic acid	FOLKERS *et al.*, (1941)
		Amino acids & peptides :	
		Hypaphorine (*S*)-form	FOLKERS, *et al.*, (1940)
		Alkaloids :	
		Erysodine & Erysovine	ABDULLAH *et al.*, (1979)
		α-Erythroidine & β-Erythroidine	AGUILAR, *et al.*, (1981)
E. arborescens	Bhutan, Burma, China, India & Nepal	**Amino acids :**	
		Hypaphorine (*S*)-form	FOLKERS et al., (1940)
		Alkaloids :	
		Erybidine, Erysodine, Erythrabine, Erythrartine, Erysotrine & *N*-Nororientaline.	GHOSAL *et al.*, (1974)
		Erysodinophorine, Orientaline (*S*)-form & Erysopinophorine	TIWARI *et al.*, (1979)
		Erysophorine	GHOSAL *et al.*, (1974)
		Erysopine, Erysosalvine, Erysotine, Erysovine, 11-Oxeorysodine, 11-Oxeorysopine & 11-Oxeorysovine	GAMES *et al.*, (1974)
		Erysotramidine & Erysotrine (+)-form	FOLKERS *et al.*, (1942)
		Erythaline, Erythramine, Erythrascine, Erythristemine &	GHOSAL *et al.*, (1974)

Erythrina	Geographical distribution	Compounds isolated	References
		β-Erythroidine & Isoerysopinophorine	MASOOD *et al.*, (1980)
E. atitlanensis	Guatemala	**Flavonoids :** Callistephin, Cyanidin, 3-sophoroside & Pelargondin 3-sophoroside	SCOGIN, (1991)
		Alkaloids : Erysodine, Erysotrine (+)-form & Erysovine.	HARGREAVES *et al.*, (1974)
E. barqueroana	Guatemala	**Alkaloids :** Erysodine, Erysovine, Erythraline (+)-form & Erythratidine	HARGREAVES *et al*, (1974)
E. berteroana	Tanzania, Carribean, Belize, Costa Rica, El Salvador, Guatemala, Mexico, Nicaragua & Panama	**Flavonoids :** Callistephin, Cyanidin 3-sophoroside & Pelargondin 3-sophoroside	SCOGIN, (1991)
		4-*O*- Methylsigmoidin	MAILLARD *et al.*, (1987)
		Amino acids & peptides : Hypaphorine (*S*)-form	FOLKERS *et al.*, (1940)
		Alkaloids : Erysodine & Erysovine	HARGREAVES *et al.*, (1974)
		α-Erythrodine & β-Erythrodine	JACKSON *et al.*, (1982)
		Erysoline, Erysonine, Erysopine, 8-Oxo-α-erythrodine & 8-Oxo-β-erythrodine	CHAWLA *et al.*, (1982)

Erythrina	Geographical distribution	Compounds isolated	References
E. x bidwilli	Tanzania, U.S.A. & Fiji	**Flavonoids :**	
		Chrysanthemin, Cyanidin 3-sophoroside & Pelargonidin 3-sophoroside	SCOGIN, (1991)
		Erythrabyssin II, Bidwillon A & B, Auriculatin & 8-γ, γ-Dimethyl-allyldaidzein	IINUMA *et al.*, (1992)
		Alkaloids :	
		Erybidine, Erysodine, Erythraline (+)-form, Erythrinine & *N*-Nororientaline	HARGREAVES *et al.*, (1974)
E. breviflora	Michoacán	**Amino acids & peptides :**	
		Hypaphorine	AGUILAR *et al.*, (1993)
		Alkaloids :	
		Erysodine, Erysovine, Erysopine, Erythravine & α-Erythroidine	AGUILAR *et al.*, (1993)
E. brucei	Ethiopia & Kenya	**Alkaloids :**	
		Crystamidine (+)-form, Erythrinine, 8-Oxoerythraline & 8-Oxoerythrinine	DAGNE *et al.*, (1984)
		Erysodine, Erysopine & Erysovine	GAMES *et al.*, (1974)
		Erythraline (+)-form, Erythratidine & 11-Methoxyerythratidine	CHAWLA *et al.*, (1985)
E. burana	Ethiopia & Kenya	**Alkaloids :**	
		Erysodine, Erysopine, Erysosalvine, Erysotine, Erysovine, Erythraline (+)-form & Erythratidine	GAMES *et al.*, (1974)
		Pterocarpans :	
		Cristacarpin	DAGNE *et al.*, (1993)

Erythrina	Geographical distribution	Compounds isolated	References
E. burtii	Kenya & Tanzania	**Alkaloids :** Erysodine & Erysovine	GAMES *et al.*, (1974)
E. caffra	Kenya, Mozambique, South Africa & India	**Flavonoids :** Callistephin, Cyanidin 3-sophoroside & Pelargondin 3-sophoroside	SCOGIN, (1991)
		Alkaloids : 10,11-Dehydroerysodine, Erysodine, Erysopine, Erysotrine (+)-form, Erysovine, Erythrinine, Erythraline (+)-form, 11-Methoxyersodine, 11-Methoxyerysopine, 11-Methoxyerysovine, 11-Methoxyerythraline, 11-Oxoerysodine & 11-Oxoerysovine	GAMES *et al.*, (1974)
E. caribaea	Belize & Mexico	**Alkaloids :** Erysodine, Erysonine, Erysopine, Erysotrine (+)-form, Erysovine, Erythaline, Erythratidine & Erythrocarine	CHAWLA *et al.*, (1985)
E. chiapasana	Guatemala & Mexico	**Flavonoids :** Callistephin, Cyanidin 3-sophroside & Pelargondin 3-sophoroside	SCOGIN, (1991)
		Alkaloids : Erysodine, Erysovine,	HARGREAVES *et al.*, (1974)

Erythrina	Geographical distribution	Compounds isolated	References
		α-Erythroidine & β-Erythroidine	
E. chiriquensis	Costa Rica, Nicaragua & Panama	**Alkaloids :** Erysodine, Erysopine, Erysovine & Erythratidine	BARAKAT *et al.*, (1977)
E. cochleata	Costa Rica & Colombia	**Alkaloids :** Erysodine, Erysoline, Erysopine, Erysotrine (+)-form, Erysovine, Erythraline, Erythratidine, Erythravine & 11-Methoxyerythratidine	CHAWLA *et al.*, (1985)
E. corralodendron	Kenya, China, India, Taiwan, Carribean, Madagascar & French Guiana	**Flavonoids :** Callistephin & Pelargonidin 3-sophoroside	SCOGIN, (1991)
		Chrysanthemin & Cyanidin 3-sophoroside	HARGREAVES *et al.*, (1974)
		Amino acids & peptides : Hypaphorine (*S*)-form	HARGREAVES *et al.*, (1974)
E. coralloides	Mexico & U.S.A.	**Flavonoids :** Callistephin, Chrysanthemin & Pelargonidin 3-sophoroside	SCOGIN, (1991)
		Alkaloids : Erysodine, Erysopine, Erysotrine (+)-form, Erysovine, Erythraline (+)-form, Erythratidine, α-Erythroidine & β-Erythroidine	HARGREAVES *et al.*, (1974)
E. costaricensis	Costa Rica, Panama, Colombia & Ecuador	**Amino acids & peptides :** Hypaphorine (*S*)-form	FOLKERS *et al.*, (1941)

Erythrina	Geographical distribution	Compounds isolated	References
		Alkaloids :	
		Erysodine, Erysonine & Erysopine	CHAWLA *et al.*, (1987)
E. crista-galli	Egypt, Ethiopia, Kenya, Mozambique, Rwanda, Sudan, Tanzania, Uganda, Zimbabwe, India, China, Taiwan, Caribbean, Belize, Costa Rica, El Salvador, Guatemala, Mexico, Panama, Mauritius, Seychelles, Argentina, Bolivia, Brazil, Paraguay & Peru	**Alliphatic natural products :**	
		Docosanoic acid, Eicosanic acid, Heptadecanoic acid, Hexadecanoic acid, Octadecanoic acid, Pentadecanoic acid & Tridecanoic acid	INGHAM *et al.*, (1980)
		Flavonoids :	
		Callistephin & Chrysanthemin,	IMAMURA *et al.*, (1981)
		Cyanidin 3-sophoroside & Pelargonidin 3-sophoroside	INGHAM *et al.*, (1980)
		Cristacarpin, 3,9-Dihydroxy-pterocarpan , Phaseollidin, Erycristagallin, Daidzein, Daidzin, Genistein & Genistin	MITSCHER *et al.*, (1987)
		Erycristin, Erythrabyssin & Sandwicensin	MITSCHER *et al.*, (1988)
		Amino acids & peptides :	
		Betaine & Hypaphorine (*S*)-form	CHAWLA *et al.*, (1987)
		Alkaloids :	
		Cristadine, Crystamidine (+)-form & *N*-Noroientaline	DEULOFEU *et al.*, (1947)
		Choline, Erysodine, Erysopine, Erysovine, Erythraline & Erythrinine	CHAWLA *et al.*, (1987)
		11-Methoxyerythraline, 11-Methoxyerythratine, 11-Methoxyerythraline *N*-oxide &	MANTLE *et al.*, (1984)

Erythrina	Geographical distribution	Compounds isolated	References
		8-Oxoerythrinine	DEULOFEU *et al.*, (1947)
		Erybidine, Erysotrine, Erythratine & Erythramine	
		Cinnamylphenols :	
		Erycristanols A, B & C	IINUMA *et al.*, (1994)
E. cubensis	Caribbean	**Alkaloids :**	
		Erysodine, Erysopine & Erysovine	FOLKERS *et al.*, (1942)
E. decora	Namibia	**Alkaloids :**	
		Erysodine, Erysopine, Erysotine, Erysovine & Erythratidine	BARAKAT *et al.*, (1977)
E. dominguezii	Argentina, Bolivia, Brazil & Paraguay	**Flavonoids :**	
		Callistephin, Chrysanthemin, Cyanidin 3-sophoroside & Pelargonidin 3-sophoroside	FOLKERS *et al.*, (1941)
		Amino acids & peptides :	
		Hypaphorine (*S*)-form	FOLKERS *et al.*, (1941)
		Alkaloids :	
		Erysodine, Erysopine & Erysovine	GENTILE *et al.*, (1942)
E. edulis	Panama, Bolivia, Colombia, Ecuador, Peru & Venezuela	**Alkaloids :**	
		Erysodine & Erysovine	WILLIAMAN *et al.*, (1970)
E. eggersii	Caribbean	**Alkaloids :**	
		Erysodine, Erysopine, Erysotine, Erythratidine & Erythravine	BARAKAT *et al.*, (1977)
E. eriotricha	Cameroon	**Simple aromatic natural products :**	
		Ferulic acid	NKENEGFACK *et al.*, (1990)

Erythrina	Geographical distribution	Compounds isolated	References
		Flavonoids :	
		Eriotriochin, Auriculatin, Dihydroauraculatin &	NKENGFACK *et al.*, (1993)
		3'-*O*-methylorobol	NKENGFACK *et al.*, (1990)
		Eriotrinol	NKENGFACK *et al.*, (1989)
		5, 4'-Dimethoxy-3'-prenylbiochanin A, Erythrinasinate, Abyssinone V, 3'-Prenylnaringenin & 2'-Hydroxy-5'-methoxybiochanin	
		Sigmoidin C, Senegalensin & Auriculatin	NKENGFAGK *et al.*, (1991)
		Eriotrichin B	NKENGFACK *et al.*, (1997)
		Sigmoidin A, Scandenone, 6, 8-Diprenylgenistein, Flemiphiilippinin B & 8-Prenyldaidzein	NKENGFACK *et al.*, (1990)
		Terpenoids :	
		Maniladiol, Serrat-14-ene-3β, 21α-diol & 28-acetoxy-erythrodiol	NKENGFACK *et al.*, (1995)
		Pterocarpans :	
		Erybraedin A, C, D & E, Isoneorautenol , Gangetinin & Calopocarpin	NKENGFACK *et al.*, (1997)
E. excelsa	Cameroon, Ivory Coast, Kenya, Mali, Nigeria, Sudan, Tanzania, Uganda, Zaire &	**Simple aromatic natural products:**	
		Hexacosyl (*E*)-ferulate	WANDJI *et al.*, (1990)
		Ferulic acid	NKENGFACK *et al.*, (1990)

Erythrina	Geographical distribution	Compounds isolated	References
	Zambia	**Flavonoids :**	
		4', 5, 7-Trihydroxy-6,8-diprenyl isoflavone	FOMUM *et al.*, (1986)
		8-Prenylluteone	NKENGFACK et al., (1989)
		Alkaloids :	
		Erysodine, Erysopine, Erysovine, Erythraline & Erythratidine, 11-Oxoerysodine & 11-Oxeoerysovine	FOLKERS *et al.*, (1942)
			HARGREAVES *et al.*, (1974)
E. falcata	Kenya, Argentina, Bolivia, Brazil, Chile, Paraguay & Peru	**Flavonoids :**	
		Callistephin, Chrysanthemin, Cyanidin 3-sophoroside & Pelargonidin 3-sophoroside	GENTILE *et al.*, (1942)
		Amino acids & peptides :	
		Hypaphorine (*S*)-form	FOLKERS *et al.*, (1940)
		Alkaloids :	
		Erysodine, Erysopine, Erysovine & Erythratidine	FOLKERS *et al.*, (1940)
E. flabelliformis	Mexico & U.S.A	**Flavonoids :**	
		Callistephin, Cyanidin 3-sophoroside & Pelargonidin 3-sophoroside	SCOGIN, (1991)
		Amino acids & peptides:	
		Hypaphorine (*S*)- form	FOLKERS *et al.*, (1940)
		Alkaloids :	
		Erysodine, Erysopine, Erysotrine & Erysovine	FOLKERS *et al.*, (1940)

Erythrina	Geographical distribution	Compounds isolated	References
E. folkersii	Belize, Guatemala, Mexico & Hawaii	**Flavonoids :**	
		Callistephin, Cyanidin 3-sophoroside & Pelargonidin 3-sophoroside	SCOGIN, (1991)
		Amino acids & peptides :	
		Hypaphorine (*S*)-form	FOLKERS *et al.*, (1940)
		Alkaloids :	
		Erysodine, Erysoline, Erysonine, Erysopine, Erysotrine, Erysovine & Erythravine & Erythraline	HARGREAVES *et al.*, (1974)
			FOLKERS *et al.*, (1940)
E. fusca	Cameroon, Ghana, Nigeria, Tanzania, Uganda, New Caledonia, Caribbean, Bolivia, Brazil, Colombia, Ecuador, Peru, Venezuela, Madagascar, Mauritius, Fiji, Samoa, Belize, Costa Rica, El Salvador, Guatemala, Honduras, Nicaragua & Panama	**Flavonoids :**	
		Cistacarpin & Pelargonidin	FOMUM *et al.*, (1986)
		Amino acids & peptides :	
		Hypaphorine (*S*)-form	FOLKERS *et al.*, (1940)
		Alkaloids :	
		Erysodine & Erysopine	FOLKERS *et al.*, (1940)
		Erysothiopine & Erysothiovine	FOLKERS *et al.*, (1944)
		Erysotrine, Erysovine, Erythraline (+)-form, Erythramine & Erythratine	HARGREAVES *et al.*, (1974)
E. gibossa	Costa Rica, Hondura, Nicaragua & Panama	**Alkaloids :**	
		Erysodine & Erysovine	HARGREAVES *et al.*, (1974)
E. glauca	Guatemala	**Pterocarpans :**	
		3-*O*-Methylcalopocarpin & Sandwicensin	MCKEE *et al.*, (1997)
E. globocalyx	Costa Rica	**Alkaloids :**	
		Erysodine, Erysopine, Erysovine, Erythraline (+)-form, α-Erythroidine	HARGREAVES *et al.*, (1974)

Erythrina	Geographical distribution	Compounds isolated	References
		& β-Erythroidine	
E. golmanii	Guatemala & Mexico	**Alkaloids :**	
		Erysodine, Erysopine, Erysotrine, Erysovine & Erythratidine	HARGREAVES *et al.*, (1974)
E. grisebachii	Caribbean	**Amino acids & peptides :**	
		Hypaphorine (*S*)-form	FOLKERS *et al.*, (1940)
		Alkaloids :	
		Erythraline	FOLKERS *et al.*, (1944)
E. guatemalensis	El Salvador, Guatemala & Honduras	**Flavanoids :**	
		Callistephin & Pelargonidin 3-sophoroside	SCOGIN, (1991)
		Alkaloids :	
		Erysodine, Erysoline, Erysonine, Erysopine, Erysotrine, Erysovine, Erythraline & Erythratidine	HARGREAVES *et al.*, (1974)
E. herbacea	Pakistan, Mexico & U.S.A	**Flavonoids :**	
		Chrysanthemin & Cyanidin 3-sophoroside	SCOGIN, (1991)
		Amino acids & peptides :	
		Hypaphorine (*S*)-form	FOLKERS *et al.*, (1940)
		Alkaloids :	
		Erybidine, Erysodine, Erysopine Erysotrine , Erythratine & *N*-Nororientaline	FOLKERS *et al.*, (1940)
E. huehuetenangenesis	Guatemala	**Alkaloids :**	
		Erysodine, Erysovine & Erythraline	HARGREAVES *et al.*, (1974)
E. humeana	Kenya, Mozambique, South Africa,	**Flavonoids :**	

Erythrina	Geographical distribution	Compounds isolated	References
	Swaziland & Zimbabwe	Callistephin & Pelargonidin 3-sophoroside	SCOGIN, (1991)
		Alkaloids :	
		10, 11-Dehydroerysodine, 10, 11-Dehydroerysovine, 11-Hydroxyerysovine, 11-Hydroxyerysodine, 11-Methoxyerysovine & 11-Methoxyerysodine	GAMES *et al.*, (1974)
		Erysodine, Erysopine & Erysovine	HARGREAVES *et al.*, (1974)
E. lanata	Indonesia & Mexico	**Alkaloids :**	
		Erysodine, Erysoline, Erysonine & Erysovine	JACKSON *et al.*, (1982)
E. lanceolata	Costa Rica, Honduras, Nicaragua & Panama	**Alkaloids :**	
		Erysodine, Erysopine, Erysovine & Erythraline	HARGREAVES *et al.*, (1974)
E. latissima	Botswana, Mozambique, South Africa, Swaziland, Zimbabwe & India	**Alkaloids :**	
		10, 11-Dehydroerysodine, 10, 11-Dehydroersovine, Erysodine, Erysopine, Erysosalvine, Erysotine, Erysovine, Erythraline Erythratidine & 11-Hydroxyerysodine	GAMES *et al.*, (1974)
E. leptorhiza	Mexico	**Alkaloids :**	
		Erysotrine, Erythraline, Erybidine, Erythratidine, Crystadimine & Erysotramidine	GARCIA-MATEOS *et al.*, (1998)

Erythrina	Geographical distribution	Compounds isolated	References
E. livingstoniana	Malawi, Mozambique & Zimbabwe	**Alkaloids :** Erysodine , Erysopine, Erysotine, Erysosalvine, Erysovine, Erythraline, Erythratidine, 11-Hydroerysodine, 11-Hydroxyerysovine, 11-Oxoerysodine & 11-Oxoerysovine	GAMES *et al.*, (1974)
E. lysistemon	Botswana, Ethiopia, Kenya, Malawi, Mozambique, South Africa, Swaziland, Tanzania, Zimbabwe, India, Sri Lanka, Australia & Mauritius	**Flavonoids :** Callistephin, Cyanidin 3-sophoroside & Pelargonidin	SCOGIN, (1991)
		Glyasperin, Licoisofavanone, 5-Deoxyglyasperin F, 5-Deoxylicoisflavanone & 2'-Hydroxyneobavaisoflavanone	MCKEE *et al.*, (1997)
		Alkaloids : 10, 11-Dehydroerysodine, 10, 11-Dehydroerysovine & Erythraline	GAMES *et al.*, (1974)
		Erysodine, Erysotrine *N*-oxide, Erythravine, 11β-Methoxyglucoery-sodine, Glucoerysodine & Rhamnoersodine	AMER *et al.*, (1991)
		Erysopine, Erysopine, Erysovine, 11-Hydroxyerysodine, 11-Hydroxyerysovine,	BARAKAT *et al.*, (1977)
		11-Methoxyerysodine & 11-Methoxyerysovine	LETCHER *et al.*, (1971) BARTON *et al.*, (1973)

Erythrina	Geographical distribution	Compounds isolated	References
		Erysotrine & 11-Methoxyerythraline Erythistemine	
E. macrophylla	El Salvador, Guatemala & Honduras	**Flavonoids :** Callistephin, Cyandin 3-sophoroside & Pelargonidin 3-sophoroside	SCOGIN, (1991)
		Amino acids & peptides : Hypaphorine (*S*)-form	FOLKERS *et al.*, (1940)
		Alkaloids : Erysodine, Erythraline, Erythrinine & Erythratidine	STAUNTON, (1979)
		11-Hydroxyerysotinone, 11-Hydroxyerythratine & 11-Hydroxyerythratidine	JACKSON *et al.*, (1982)
		Erysopine, Erysotrine & Erysovine	HARGREAVES *et al.*, (1974)
E. melancantha	Ethiopia, Kenya, Socotra, Somalia & Tanzania	**Alkaloids :** Erymelanthine & Erysovine	DAGNE *et al.*, (1984)
E. merriliana	New Guinea	**Alkaloids :** Erysodine, Erysopine & Erysovine	BARAKAT et al., (1977)
E. mexicana	Guatemala & Mexico	**Flavanoids :** Callistephin, Cyanidin 3-sophoroside & Pelargonidin 3-sophoroside	SCOGIN, (1991)
		Alkaloids : Erysotrine, Erythraline, Erybidine, Erythratidine, Crystamidine & Erysotramidine	GARCIA-MATEOS *et al.*, (1998)

Erythrina	Geographical distribution	Compounds isolated	References
E. mildbraedi	Cameroon, Ghana, Guinea, Ivory Coast, Liberia, Nigeria, Sierra Leone, Togo, Uganda, Zaire	**Simple aromatic natural products :**	
		Erythrinasinate	FOMUM *et al.*, (1986)
		Flavonoids :	
		Erybraedin A, B & C, Erythrabyssin II & Isoneorautenol	MITSCHER *et al.*, (1988)
		Erybraedin D & E	MITSCHER et al., (1988)
		Alkaloids :	
		Erysodine, Erysopine & Erythraline	BARAKAT *et al.*, (1977)
E. oaxacana	Mexico	**Alkaloids :**	
		Erysotrine, Erythraline, Erybidine, Erythratidine, Crystamidine & Erysotramidine	GARCIA-MATEOS *et al.*, (1998)
E. oliviae	Mexico	**Alkaloids :**	
		Erysodine, Erysovaline, Erysovine, Erysotine, Erysotrine, Erythraline & Erythartidine	HARGREAVES *et al.*, (1974)
E. orophila	Mexico	**Alkaloids :**	BARAKAT *et al.*, (1977)
		Erysodine, Erysopine, Erysotine, Erysovine & Erythratidine	
E. pallida	Mauritius & Venezuela	**Flavonoids :**	
		Cyanidin & Pelargonidin	
		Amino acids & peptides :	
		Hypaphorine (*S*)-form	FOLKERS *et al.*, (1940)
		Alkaloids :	
		Erysodine, Erysopine, Erysothiovine	FOLKERS *et al.*, (1942)

Erythrina	Geographical distribution	Compounds isolated	References
		& Erysovine	
E. perrieri	Comoro Is & Madagascar	**Alkaloids :**	
		Erysodine, Erysopine, Erysovine, Erythraline & Erythrtidine	GAMES *et al.*, (1974)
E. poeppigiana	Kenya, Nigeria, Sao Tome, Sierra Leone, Tanzania, Uganda, India, Indonesia, Caribbean, Costa Rica, El Salvador, Guatemala, Panama, Bolivia, Brazil, Colombia, Ecuador, Peru, Venezuela	**Flavonoids :**	
		Pelargonidin	FOLKERS *et al.*, (1940)
		Amino acids & peptides :	
		Hypaphorine (*S*)-form	FOLKERS *et al.*, (1940)
		Alkaloids :	
		Dehydro-α-erythroidine, Erybidine, Erysodine, Erysopine, Erysotrine, Erythratidine, Erythratidinone, 11-Hydroxyerythratidine, 11-Hydroxy-*epi*-erythratidine & Isoboldine (*S*)-form	JACKSON *et al.*, (1982)
		Erysothiovine	FOLKERS *et al.*, (1944)
		Erysovine	HARGREAVES *et al.*, (1974)
		α-Erythriodine, β-Erythriodine & *N*-Nororientaline	BARTON *et al.*, (1973)
E. pudica	Mexico	**Flavonoids :**	
		Chrysanthemin & Cyanidin 3-sophoroside	SCOGIN, (1991)
E. resupinata	India	**Alkaloids :**	
		Erysodine, Erysopine & Erysovine	BARAKAT *et al.*, (1977)
E. rubrinervia	Panama, Bolivia, Colombia, Ecuador, Peru, Venezuela	**Amino acids & peptides :**	
		Hypaphorine (*S*)-form	FOLKERS *et al.*, (1941)

Erythrina	Geographical distribution	Compounds isolated	References
		Alkaloids :	
		Erysodine, Erysopine, Erysovine & Erythratidine	JACKSON *et al.*, (1982)
E. salviflora	Guatemala	**Alkaloids :**	
		Erysodine, Erysoflorinone, Erysopine, Erysosalvine, Erysosalvinone, Erysotine, Erysotinone, Erysovine & Erythratidine	HARGREAVES *et al.*, (1974)
		α-Erythroidine & β-Erythroidine	JACKSON *et al.*, (1982)
E. sandwicensis	Hawaii	**Flavonoids :**	
		Cristacarpin, Demethylvestitol, 3, 9-Dihydroxypterocarpan, Glycinol, Isovestinol, Phaseollidin, Sandwicarpin & Sandwicesin	INGHAM *et al.*, (1980)
		Alkaloids :	
		Erythramine	FOLKERS *et al.*, (1939)
		Erysodine, Erysopine & Erysovine	FOLKERS *et al.*, (1940)
E. senegalensis	Benin, Cameroon, Chad, Gambia, Ghana, Guinea, Ivory Coast, Liberia, Mali, Niger, Nigeria, Senegal, Sierra Leone, Togo & Mauritius	**Simple aromatic products :**	
		Defuscin & Octacosyl (*E*)-ferulate	WANDJI *et al.*, (1990)
		Erythrinasinate	FOMUM *et al.*, (1986)
		Flavonoids :	
		Auriculatin, 2, 3-Dihydroaurisulatin & 4', 5, 7-Trihydroxy-6, 8-diprenyl isoflavone	TAYLOR *et al.*, (1986)
		Cajaflavanone (*S*)-form & Scandenone	FOMUM *et al.*, (1986)
			FOMUM *et al.*, (1987)

Erythrina	Geographical distribution	Compounds isolated	References
		Lonchocarpol A (*S*)-form, 5, 4'-dihydroxy-8-(γ, γ-dimethylallyl)-5"-(hydroxyisopropyl)dihydro-furano (2", 3") isoflavone & Senegalensin	FOMUM *et al.*, (1987)
		Erysenegalensein B & C	WANDJI *et al.*, (1990)
		Erythrisenegalone	WANDJI *et al.*, (1995)
		Senegalensein	FOMUM *et al.*, (1985)
		Lupinifolin & Erysenegalensein H & I	FOMUM *et al.*, (1987)
		Erysenegalensein J & K	
		Erysenagalensein L & M	WANDJI *et al.*, (1993)
		Amino acids & peptides :	WANDJI *et al.*, (1995)
		Hypaphorine (*S*)-form	
		Alkaloids :	WANDJI *et al.*, (1995)
		Erysodine & Erysopine	
		Erysotrine, Erysovine, Erythraline,	FOLKERS *et al.*, (1941)
		Erythratidine, 11-Hydroxyerysodine,	GAMES *et al.*, (1974)
		11-Hydroxyerysovine,	
		11-Oxoerysodine &	
		11-Oxoerysovine	
		Glucoerysodine	
		Terpenoids :	WANDJI *et al.*, (1995)
		β-amyrin, Maniladiol,	
		Erythrodiol, Oleanoic acid	NKENGFACK *et al.*, (1995)
		& Cornulacic acid	

Erythrina	Geographical distribution	Compounds isolated	References
E. sigmoeidia	Cameroon, Chad, Guinea, Ivory Coast, Mali, Nigeria, Senegal, Sudan & Togo	**Simple aromatic natural products :**	
		Erythrinasinate	NKENGFACK *et al.*, (1989)
		Flavonoids :	
		Abyssinone IV, Sigmoidin F & 5, 7, 4'-Trihydroxy-3'-methoxy-5'-(1"-prenyl) flavanone	PROMSATTHA *et al.*, (1989)
		Abyssinone V, Licoflavanone & 2', 5, 7-Trihydroxy-4', 5'-dimethoxy isoflavone	NKENGFACK *et al.*, (1989)
		Auriculatin, 8-Prenylluteone, Scandenone & 3', 4', 5, 7-Tetrahydroxy-6, 8-diprenyl isoflavone	NKENGFACK *et al.*, (1989)
		2, 3-Dihydroauriculatin, Eriotriochin & 4', 5, 7-Trihydroxy-3'-methoxyisflavone	NKENGFACK *et al.*, (1990)
		Sigmoidin E	PROMSATTHA *et al.*, (1988)
		3', 7-Dihydroxy-4', 5-dimethoxy-5'-preylisoflavone	
		Sigmoidin A, B & C	NKENGFACK *et al.*, (1990)
		Sigmoidin D	FOMUM *et al.*, (1986)
		Sigmoidin G	PROMSATTHA *et al.*, (1986)
		Sigmoidin I, Corylin & Neobavaisoflavone	NKENGFACK *et al.*, (1993)
		Sigmoidin H, Abyssinone VI, Phaseollin & Erythrabyssin II, Neobavaisoflavone & 6, 8-diprenylgenstein	NKENGFACK et al., (1994)
			NKENGFACK *et al.*, (1994)
			FOMUM *et al.*, (1985)

Erythrina	Geographical distribution	Compounds isolated	References
		Terpenoids :	
		28-Acetylerythrodiol, Maniladiol & Serratenediol	NKENGFACK *et al.*, (1990)
		Sigmoisides A & B	KOUAM *et al.*, (1991)
		Sigmoisides C & D	MBAFOR *et al.*, (1997)
		Pterocarpans :	
		Phaseollidin	NKENGFACK *et al.*, (1994)
		Steroids :	
		Stigmasterol	FOMUM *et al.*, (1986)
		Soyasapogenol-B &	MBAFOR *et al.*, (1997)
		3-*O*-(β-D-glucopyranosyl)-sitosterol	GAMES *et al.*, (1974)
		Alkaloids :	
		Erysodine, Erysopine, Erysovine & Erythraline	
E. smithiana	Ecuador	**Alkaloids :**	
		Erysodine & Erysovine	WILLIAMAN *et al.*, (1970)
E. sousae	Mexico	**Alkaloids :**	
		Erysotrine, Erythraline, Erybidine, Erythratidine, Crystamadine & Erysotramidine	GARCIA-MATEOS *et al.*, (1998)
E. sp 2	Costa Rica	**Alkaloids :**	
		Erysodine, Erysotrine, Erysovine, Erythraline, α-Erythriodine & β-Erythriodine	HARGREAVES *et al.*, (1974)
E. speciosa	Egypt, Brazil & India	**Flavonoids :**	
		Callistephin, Cyanidin 3-	SCOGIN, (1991)

Erythrina	Geographical distribution	Compounds isolated	References
		sophoroside & Pelargonidin 3-sophoroside	
		Alkaloids :	
		Erysodine & Erysovine	HARGREAVES *et al.*, (1974)
E. standleyana	Belize, Guatemala & Mexico	**Alkaloids :**	
		Erysodine, Erysopine, Erysovine, α-Erythroidine & β-Erythroidine	HARGREAVES *et al.*, (1974)
E. stricta	Burma, Cambodia, China, India, Laos, Nepal, Thailand & Vietnam	**Aliphatic natural products :** 1-Dotriacontanol, Heptacosane, 1-Heptatriacontanol, Hexacosanoic acid, 1-Hexatriacontanol, 8-(15-Hydroxypentadecyl)-7-methoxy-2*H*-benzopyran-2-one, Octacosanoic acid, Octadecane, 1-Octatriacontanol, 1-Pentatriacontanol, Tetracosanoic acid & Tritriacontane	SINGH *et al.*, (1981)
		Steroids :	
		β-Sitosterol & Stigmasterol	SINGH *et al.*, (1981)
		Amino acids & peptides :	
		Hypaphorine (*S*)-form	SINGH *et al.*, (1981)
		Alkaloids :	
		10, 11-Dehydroerysodine, 10, 11-Dehydroerysovine, Erysodine, Erysopine, Erysovine,	GAMES et al., (1974)

Erythrina	Geographical distribution	Compounds isolated	References
		Erythraline, Erythrinine, 11-Hydroxyerysodine & 11-Hydroxyerysodine	
E. suberosa	Bhutan, Burma, Cambodia, India, Nepal, Pakistan, Thailand & Vietnam	**Aliphatic natural products :** Docosanoic acid, Eicosanoic acid, Hexadecanoic acid, Octadecanoic acid & Tetradecanoic acid	SINGH *et al.*, (1970)
		Hexacosanoic acid, 1-Hexacosanol, Octacosanol, 1-Octacosanlol & Tetradecanoic acid	SINGH *et al.*, (1975)
		Flavonoids : Cyanin, Delphin & Pelargonin	SINGH *et al.*, (1975)
		3', 4', 5, 7-Tetrahydroxy-2'-prenylflavanone	SINGH *et al.*, (1981)
		Steroids : Campesterol, Cholesrerol, Stigmasterol & β-Sitosterol	CHAUHAN *et al.*, (1987)
		Amino acids & peptides : Hypaphorine (*S*)-form	SINGH *et al.*, (1981)
		Alkaloids : Erysodine, Erysopine, Erythraline, Erythratidine, Erythrinine, 8-Oxoerythrinine, 11-Methoxyerythraline & 11β-Methoxyerythraline	SINGH *et al.*, (1981)
		Erysotrine	CHAWLA *et al.*, (1985)
			MIANA *et al.*, (1972)
E. subumbrans	Burma, India, Java, Laos, Moluccas, Malaysia, Sri	**Amino acids & peptides :** Hypaphorine (*S*)-form	FOLKERS *et al.*, (1939)

Erythrina	Geographical distribution	Compounds isolated	References
	Lanka, Phillippines, Sulawesi, Sumatra, Thailand, Vietnam, Mauritius, Fiji & Samoa	**Alkaloids :** 2-Epierythratine, Erysodine, Erysoline, Erysopine, Erysovine, Erythratine & Erysoflorinone	GUNATILAKA *et al.*, (1978)
		Erythramine	FOLKERS *et al.*, (1939)
E. sykesii	Australia & New Zealand	**Flavonoids :** Callistephin, Cyanidin 3-sophoroside & Pelargonidin 3-sophoroside	SCOGIN, (1991)
E. tahitensis	Society Is	**Alkaloids :** Erysodine, Erysopine, Erysovine, Erythraline, Erythratidine, 11-Oxoerysodine, 11-Oxoerysopine & 11-Oxoerysovine	GAMES *et al.*, (1974)
E. tajumulcensis	Guatemala & Mexico	**Flavonoids :** Callistephin, Cyanidin 3-sophoroside & Pelargonidin 3-sophoroside	SCOGIN, (1991)
		Alkaloids : Erysodine, Erysopine, Erysotrine, Erysovine & Erythraline	HARGREAVES *et al.*, (1974)
E. tholloniana	Gabon, Nigeria & Zaire	**Alkaloids :** Erysodine, Erysoline, Erysopine, Erysotine, Erysovine, α-Erythroidine & β-Erythroidine	CHAWLA *et al.*, (1985)

Erythrina	Geographical distribution	Compounds isolated	References
E. variegata	Egypt, Nigeria, Sao Tome, Principe, Senegal, Sudan, Tanzania, Uganda, Burma, Cambodia, China, Hong Kong, India, Indonesia, Laos, Malaysia, Phillippines, Sri Lanka, Taiwan, Thailand, Vietnam, Australia, New Caledonia, New Guinea, Caribbean, Belize, El Salvador, Madagascar, Maldives, Mauritius, Seychelles, Fiji, Samao, Tonga & Brazil	**Aliphatic natural products :**	
		Docosanoic acid, Eicosanoic acid, Hexadecanoic acid, Octadecanoic acid & Tetradecanoic acid,	SINGH *et al.*, (1981)
		1-Docosanol, Hexacosanoic acid, Octacosnoic acid, 1-Octacosanol & 1-Tetracosanol	SINGH *et al.*, (1975)
		Carbohydrates :	
		Rutin	DESHPANDE *et al.*, (1977)
		Simple aromatic natural products :	
		Caffeic acid, Ferulic acid, Dihydrooxyresveratrol & Oxyresveratol	DESHPANDE *et al.*, (1977)
		Flavonoids :	
		Alpinumisoflavone, Erythrinin A & C, Osajin, Wighteone & Erycristagallin,	DESHPANDE et al., (1977)
		Erythrabyssin II, Eryvariestyrene, Isobavachin, Phaeollidin, Phaseollin, Scandenone & 4', 5, 7-Trihydroxy-6, 8-diprenylisoflavone	TELIKEPALLI *et al.*, (1990)
		Isorhamnetin 3-rhamnosylgluc-oside, Quercetin, Rutin & Quercetin 3-*O*-(rhamnosylglucoside) 4'-glucoside	SINGH *et al.*, 1975

Erythrina	Geographical distribution	Compounds isolated	References
		Pelargonidin	SCOGIN, 1991
		Steroids :	
		Campersterol, 24-Methylene-lophenol, α-Sitosterol & Stigmasterol	SINGH *et al.*, (1981)
		Amino acids & peptides :	
		Hypaphorine (*S*)-form, Stachydrine (*S*)-form, Tryptophan (*S*)-form, *N, N*-Dimethyltryptophan & *N, N*-Dimethyltryptophan methociation methyl ester	GHOSAL *et al.*, (1972)
		Alkaloids :	
		Coreximine, Erybidine, Scoulerine & Reticuline	ITO *et al.*, (1970)
		Erysodine, Erysopine, Erythraline, Erythramine, Erythratine, β-Erythriodine, Norprotosinomenine & Protosinomenine	GHOSAL *et al.*, (1971)
		Choline, Erysodienone, Erysonine, Erysopitine, Erysotrine & Erysovine	GHOSAL *et al.*, (1972)
		2-Epierythratidine, Erysotine, Erythratidine & 11-Hydroxy-*epi*-erythratidine	CHAWLA *et al.*, (1988)
		3-Demethoxyerythratidinone & Erythatidinone	BARTON *et al.*, (1973)
		Erythrartine	EL-OLEMY *et al.*, (1978)
		Erythratinone & Erythrinine	FOLKERS *et al.*, (1940)

Erythrina	Geographical distribution	Compounds isolated	References
		N-Nororientaline	ITO *et al.*, (1970)
		Stachydrine (*S*)-form & Erythritol	CHAWLA *et al.*, (1993)
E. velutina	Uganda, Sri Lanka,	**Flavonoids :**	FOLKERS *et al.*, (1940)
	Caribbean, Brazil, Colombia,	Pelargondin	
	Ecuador, Peru & Venezuela	**Amino acids & peptides :**	FOLKERS *et al*, (1940)
		Hypaphorine (*S*)-form	
		Alkaloids :	FOLKERS *et al*, (1940)
		Erysodine, Erysovine & Erythraline	
E. verna	Bolivia & Brazil	**Amino acids & peptides :**	
		Hypaphorine (*S*)-form	SARRAGIOTO *et al.*, (1981)
		Alkaloids :	
		Erysotrine, Erysotrine *N*-oxide,	SARRAGIOTO *et.al.*, (1981)
		Erythratine & Erythratine *N*-oxide	
E. vespertilio	Australia	**Alkaloids :**	
		Erysodine, Erysopine, Erysovine, Erythraline, Erythrinine &	GAMES *et al.*, (1974)
		11-Methoxyerythraline	
E. zeyheri	Botswana, Lesotho, South	**Alkaloids :**	
	Africa & Zimbabwe	Erysodine, Erysotrine, Erysovine,	GAMES *et al.*, (1974)
		Erythraline & 11-Oxoerythraline	

2.3 TRADITIONAL USES AND BIOLOGICAL ACTIVITY OF THE GENUS *Erythrina*

A. Traditional uses

Table 2.3.1 Traditional uses of *Erythrina* species

Species	Plant part used	Administration	Reference
E. abyssinica	Bark	Burnt ash used on wounds. Used to treat elephantitis.	KOKWARO, 1987 ICHIMARU *et al.*, 1996
	Roots	Used to treat malaria and syphilis.	ICHIMARU *et al.*, 1996
E. breviflora	Flowers	Used for insomnia.	AGUILAR *et al.*, 1993
E. caffra	Leaves	Paste used for urinary complaints and venereal diseases. Infusion used for earache.	HUTCHINGS *et al.*, 1996 VAN RENSBURG, 1982
	Bark	Used for sprains.	VAN RENSBURG, 1982
E. costaricensis	Leaves	Infusion used for menstrual pain.	VON REIS *et al.*, 1982
E. humeana	Roots	Used for sprains, tuberculosis and bronchitis.	VAN RENSBURG, 1982
	Bark	Burnt ash put onto umbilical cord of new born babies.	VAN RENSBURG, 1982
E. lanceolota	Leaves	Tea used for kidney problems.	VON REIS *et al.*, 1982
E. latissima	Bark	Ground to a powder and put onto open sores.	VAN RENSBURG, 1982
E. lysistemon	Leaves	Clears wounds of maggots. Used in childbirth. Poultice used for abscesses and swellings.	VAN RENSBURG, 1982 ROBERTS, 1990 HUTCHINGS *et al.*, 1996
	Bark	Used for toothache. Ash of burnt bark used to disinfect wounds. Strips used on sore hands. Burnt formentation used on sprains and strained ligaments.	ROBERTS, 1990 ROBERTS, 1990 ROBERTS, 1990 VAN RENSBURG, 1982
E. poeppigiana	Bark	Paste used on strained ligaments.	VON REIS *et al.*, 1982
E. sacleuxii	Roots	Used for gonorrhea.	VON REIS *et al.*, 1982
E. senegalensis	Leaves	Used for gonorrhea and stomach pains.	WANDJI *et al.*, 1995
E. sigmoidea	Bark	Used to treat syphilis, wounds and ulcers. Used to treat female sterility. Used to treat gonorrhea.	MBAFOR *et al.*, 1997 MBAFOR *et al.*, 1997 NKENGFACK *et al.*, 1994

Species	Plant part used	Administration	Reference
E. variegata	Seeds	Used for asthma	CHAWLA *et al.*, 1993
	Bark	Inflammation and viruses. Used as an antiseptic. Used for the treatment of liver disorders.	COX *et al.*, 1994 TELIKEPALLI *et al.*, 1990 TELIKEPALLI *et al.*, 1990
	Leaves	Relief of pain in joints. Used as a diuretic.	TELIKEPALLI *et al.*, 1990 TELIKEPALLI *et al.*, 1990
E. zeyheri	Seeds	Used for asthma.	VAN RENSBURG, 1982
	Leaves	Tea used for tuberculosis.	VAN RENSBURG, 1982
	Bark	Ground to a powder and used for rheumatism. Tea used for blood disorders.	VAN RENSBURG, 1982 VAN RENSBURG, 1982

B. Biological activity

Most of the studies carried out on the genus *Erythrina* have been chemical studies. It is only recently with increased realization of the importance of ethnobotany that researchers have started testing these plants for biologically active compounds. Table 2.3.2 shows the biologically active compounds that have been isolated. From Table2.3.2 it is noticeable that no biologically active compounds have been isolated from *Erythrina* species indigenous to South Africa.

Table 2.3.2 Biologically active compounds isolated from *Erythrina* species

Species	Isolated compound	Biological activity	
E. berteroana	5, 7-Dihydroxy-3-(5-hydroxy-4-methoxy-3-(3-methyl-2-butenyl)phenyl-2, 3-dihydro-4*H*-1-benzopyran-4-one	Anti-fungal activity against *Cladosporium cucumerinum.*	MAILLARD *et al.*, 1988
E. x bidwilli	Bidwillon B & Auriculatin	Anti-bacterial activity against *Fuscobacterium nucleatum* & *Prevotella intermedia.*	IINUMA *et al.*, 1992
E. excelsa	Auriculatin, *n*-octaco- sanyl-4-hydroxy-3-methoxycinnamate & 6,3-diprenyl- genistein	Anti-bacterial activity against *Staphylococcus aureus.*	KOUAM *et al.*, 1991
E. eriotricha	Eriothrin & Erybraedins A & C	Anti-bacterial activity against *Staphylococcus aureus.*	NKENGFACK *et al.*, 1995
	Auriculatin, *n*-octacosanyl-4-hydroxy-3-methoxycinnamate & 6,3-diprenylgenistein	Anti-bacterial activity against *Staphylococcus aureus.*	KOUAM *et al.*, 1991
E. glauca	3-*O*-Methylcalopocarpin & Sandweicin	Anti-HIV activity.	MCKEE *et al.*, 1997
E. senegalensis	Auriculatin, *n*-octaco- sanyl-4-hydroxy-3-meth-oxycinnamate & 6,3-diprenylgenistein	Anti-bacterial activity against *Staphylococcus aureus.*	KOUAM *et al.*, 1991
E. sigmoidea	Erythrabassin II	Anti-bacterial activity against *Staphylococcus aureus.*	NKENGFACK *et al.*, 1994
	Sigmoidin A & B	Anti-fungal activity against *Aspergillus fumigatus.*	NKENGFACK *et al.*, 1994
		Anti-bacterial activity against *Staphylococcus aureus* & *Micrococcus luteus*	BIYITI *et al.*, 1988
E. variegata	4'-hydroxy-3',5'-diprenyl, 3,9-dihydroxy-2,10-diprenyl pterocarp-6a-ene and 4'-hydroxy-3',5', 6-triprenyl	Anti-inflammatory activity	COX, 1995

2.4 AIMS

The aims of this study was basically ;

1. To carry out a phytochemical analysis on the bark and leaves of *Erythrina* species indigenous to South Africa;
2. To determine if the bark and leaves of the species indigenous to South Africa displayed any anti-inflammatory and anti-bacterial activity;
3. To extract and isolate the putative anti-inflammatory and anti-bacterial compounds; and
4. To attempt identification of the active compounds.

CHAPTER THREE
PHYTOCHEMICAL ANALYSIS

3.1. INTRODUCTION

Plants are solar powered chemical factories that produce both primary and secondary metabolites. The primary metabolites are compounds needed for growth and physiological processes. Secondary metabolites have no obvious roles in a plants primary metabolism. They are non-nutritive and are not directly essential for growth. It is thought that these compounds play ecologically significant roles as :

i. Pollinator attractants,

ii. Defensive and protective mechanisms, and

iii. Allelochemicals.

Secondary metabolites are biosynthetically derived from primary metabolites and are more limited in their occurrence and distribution. They are generally metabolically expensive to produce and are usually present in much smaller quantities than primary metabolites.

Some of the types of secondary metabolites found in *Erythrina* are (Table 2.2.1) :

i. **Alkaloids -** These compounds are derivatives of tertiary amines while others contain primary, secondary and quaternary nitrogen (WAGNER, BLADT & ZGAINSKI, 1984);

ii. **Flavonoids -** These compounds are typical phenolic compounds and are also conjugated aromatic componds (SWAIN, 1986). The various types of flavonoids differ in their degree of oxidation of the C ring and in the substitution of the A and B rings (WAGNER, BLADT & ZGAINSKI, 1984);

iii. **Coumarins -** A common feature of these compounds is the presence of isoprenoid chains attached to a carbon or an oxygen atom of the nucleus. The prenyl group is often found as an epoxide (MURRAY, MÉNDEZ & BROWN, 1982); and

iv.**Triterpenes-** These are derivatives of plant sterols (GOODWIN, 1973).

3.2 MATERIALS AND METHODS

A. Extraction of plant material

Plant material (bark and mature leaves) of *E. caffra, E. humeana, E. latissima, E. lysistemon* and *E. zeyheri* was collected from the Botany Departmental Garden at the University of Natal, Pietermaritzburg during February 1998. The plant material was dried in an oven at 50 ^{0}C and stored at room temperature in brown paper bags (bark material was not available for *E. zeyheri*). One gram of finely ground plant material was extracted individually with 10 ml ethanol in an ultrasound bath for 30 min. The extracts were filtered and dried. The residues were resuspended in ethanol to a final concentration of 25 mg ml^{-1}.

B. Thin layer chromatography

Twenty µl of each of the plant extracts (25 mg ml^{-1}) were streaked as 1 cm bands onto five 20x10 cm plastic Merck silica gel TLC plates. All plates were run in hexane : ethyl acetate, 2 : 1 (v/v) to obtain separation of compounds. The plates were viewed under normal and ultraviolet light, 254 and 366 nm. Plates were also sprayed with Dragendorff reagent (Solution A - 0.85 g basic bismuth nitrate dissolved in 10 ml glacial acetic acid and 40 ml water under heating. Solution B - 8 g potassium iodide dissolved in 30 ml water. Solutions A and B are mixed in a 1 : 1 ratio) for detection of alkaloids, fast blue (3, 3'-Dimethoxybiphenyl-4, 4'-bis(diazonium)-dichloride) reagent (0.5 g fast blue salt dissolved in 100 ml water) for detection of flavonoids, potassium hydroxide reagent (5% or 10% ethanolic potassium hydroxide) for detection of coumarins and anisaldehyde-sulphuric acid reagent (0.5 ml anisaldehyde mixed with 10 ml glacial acetic acid) to detect triterpenes (WAGNER, BLADT & ZGAINSKI, 1984).

3.3 RESULTS AND DISCUSSION

From Figure 3.3.1 it is evident that there are a number of compounds that are similar in the leaf extracts amongst the species. There are a few differences amongst the species but generally the leaf extracts appear to have similar profiles when viewed under normal light. Under normal light very few compounds are visible in the bark extracts. This is probably because bark contains fewer pigments than leaves.

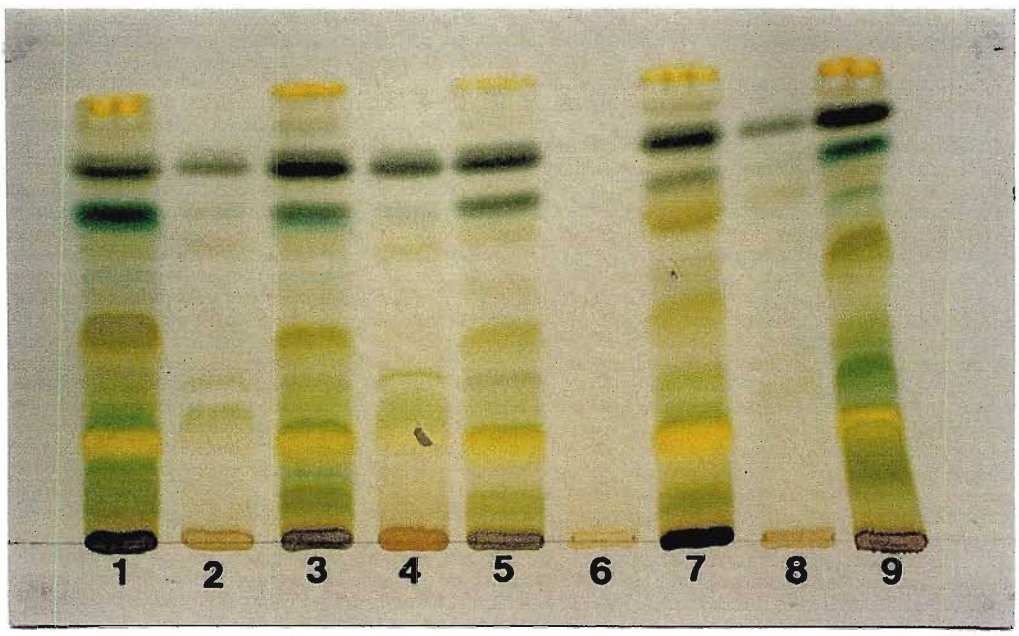


Figure 3.3.1 TLC plate of leaf and bark ethanolic extracts under normal light. Lanes 1, 3, 5, 7 and 9 are the leaf extracts of *E. caffra*, *E. humeana*, *E. latissima*, *E. lysistemon* and *E. zeyheri*, respectively. Lanes 2, 4, 6 and 8 are the bark extracts of *E. caffra*, *E. humeana*, *E. latissima* and *E. lysistemon*, respectively.

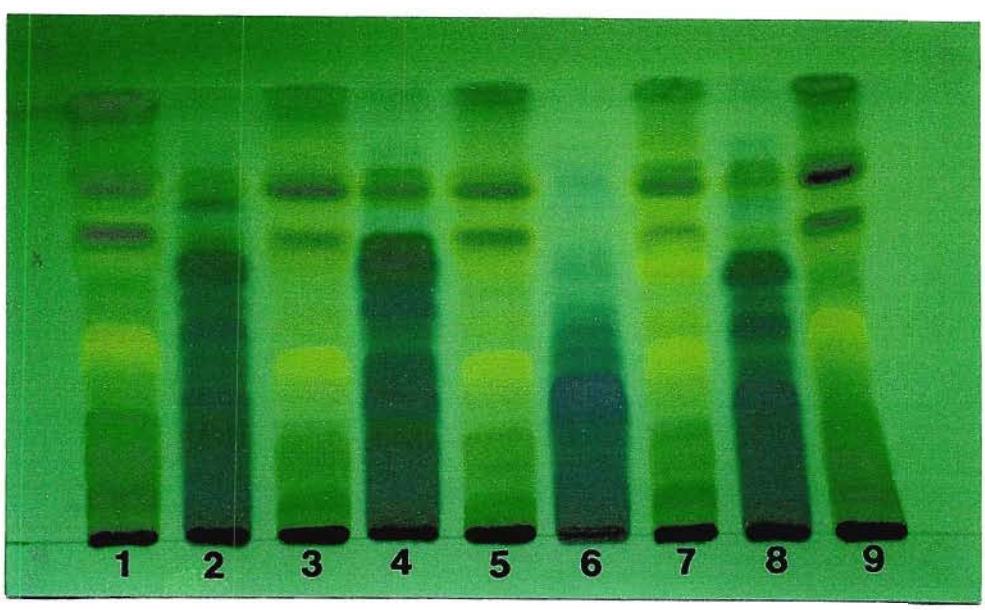


Figure 3.3.2 TLC plate of leaf and bark ethanolic extracts under ultraviolet light, (254 nm). Lanes 1, 3, 5, 7 and 9 are the leaf extracts of *E. caffra*, *E. humeana*, *E. latissima*, *E. lysistemon* and *E. zeyheri*, respectively. Lanes 2, 4, 6 and 8 are the bark extracts of *E. caffra*, *E. humeana*, *E. latissima* and *E. lysistemon*, repectively.

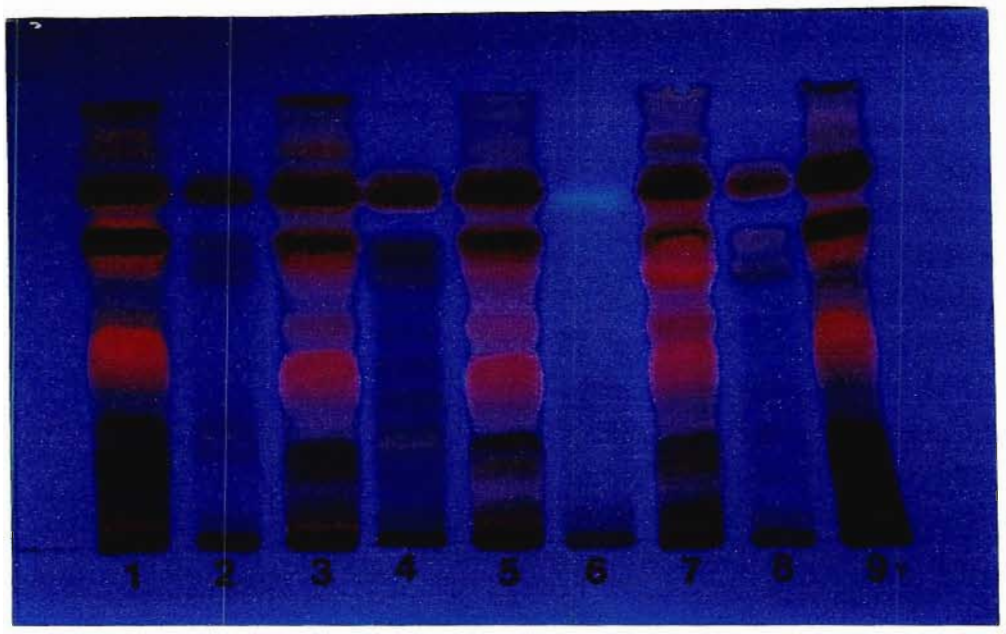


Figure 3.3.3 TLC plate of leaf and bark ethanolic extracts under ultraviolet light, (366 nm). Lanes 1, 3, 5, 7 and 9 are the leaf extracts of *E. caffra*, *E. humeana*, *E. latissima*, *E. lysistemon* and *E. zeyheri*, respectively. Lanes 2, 4, 6 and 8 are the bark extracts of *E. caffra*, *E. humeana*, *E. latissima* and *E. lysistemon*, respectively.

The bark extracts appear to contain compounds that are more visible under ultraviolet light; (254 and 366 nm) (Figures 3.3.2 and 3.3.3) than normal light. The banding pattern of the bark extract of *E. latissima* differs a lot from the banding patterns of the bark extracts of the other species. There seems to be a great similarity in the banding patterns of *E. caffra*. and *E. lysistemon* in both the bark and leaf extracts when viewed under ultraviolet light.

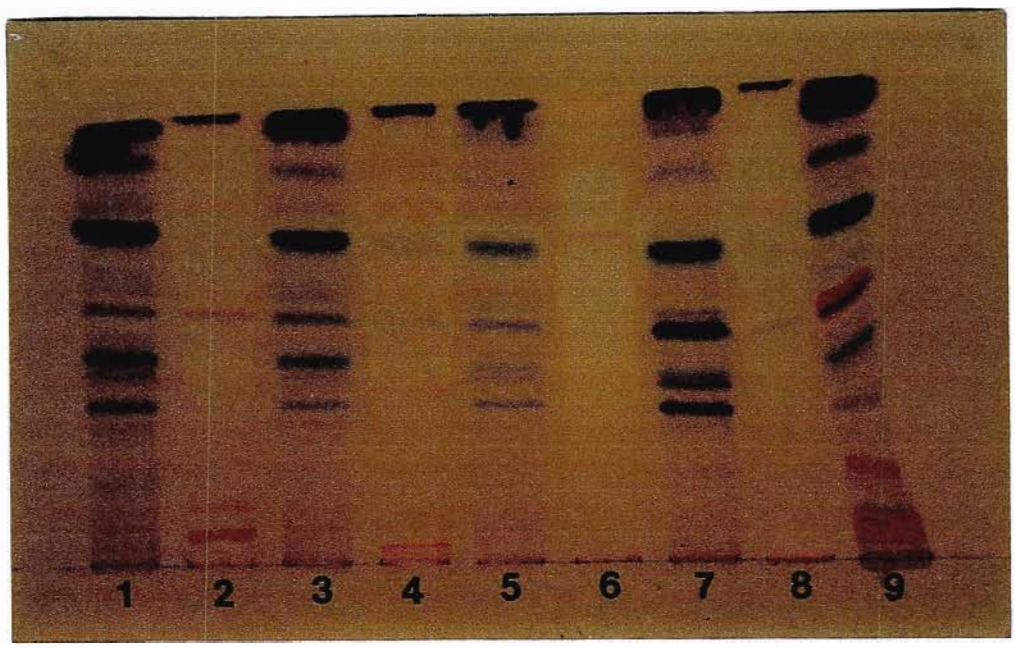


Figure 3.3.4 TLC plate of leaf and bark ethanolic extracts stained with Dragendorff reagent. Orange bands indicate the presence of alkaloids. Lanes 1, 3, 5, 7 and 9 are the leaf extracts of *E. caffra*, *E. humeana*, *E. latissima*, *E. lysistemon* and *E. zeyheri*, respectively. Lanes 2, 4, 6 and 8 are the bark extracts of *E. caffra*, *E. humeana*, *E. latissima* and *E. lysistemon*, respectively.

This genus is known for its alkaloids. As can be seen in Table 2.2.1 such compounds are present in the species analyzed. Most of the known alkaloids have been isolated from the seeds, roots and flowers of these plants. From the orange bands in Figure 3.3.4 it is clear that there are a number of alkaloids in both bark and leaf extracts of all five the species tested. The leaf extract of *E. zeyheri* appears to contain the most alkaloids. Again there seems to be a similar banding pattern amongst the species where the alkaloids seem to be found at or close to the baseline.

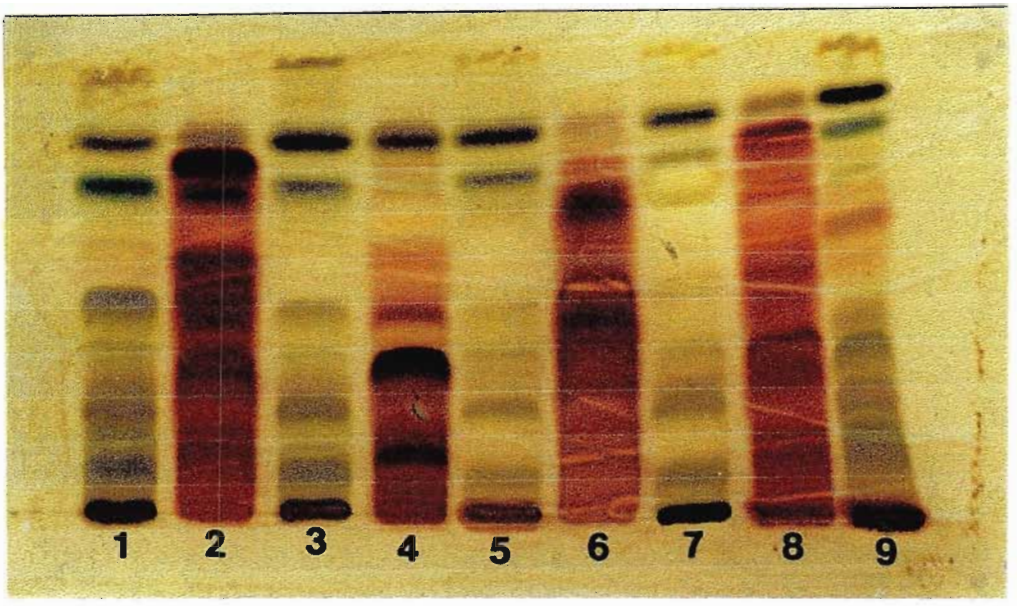


Figure 3.3.5 TLC plate of leaf and bark extracts stained with fast blue (3, 3'-Dimethoxybiphenyl-4, 4'-bis(diazonium)-dichloride) reagent. Purple bands indicate the presence of flavonoids. Lanes 1, 3, 5, 7 and 9 are the leaf extracts of *E. caffra*, *E. humeana*, *E. latissima*, *E. lysistemon* and *E. zeyheri*, respectively. Lanes 2, 4, 6 and 8 are the bark extracts of *E. caffra*, *E. humeana*, *E. latissima* and *E. lysistemon*, respectively.

From Figure 3.3.5 it is clear that the leaf extracts contained very little or no flavonoids. The bark extracts seem to have a large number of flavonoids. Isolation from the bark (Lanes 2 and 8) would therefore appear to be a viable venture. The bark extracts of *E. caffra* and *E. lysistemon* appear to have similar banding patterns. The flowers of these two species contain the same flavonoids (Table 2.2.1) it is therefore likely that the bark extracts of the two species could contain the same flavonoids. *E. latissima* and *E. zeyheri* appear to have flavonoids as there is purple discolouration at the baseline but no flavonoids have ever been isolated from these species (Table 2.2.1).

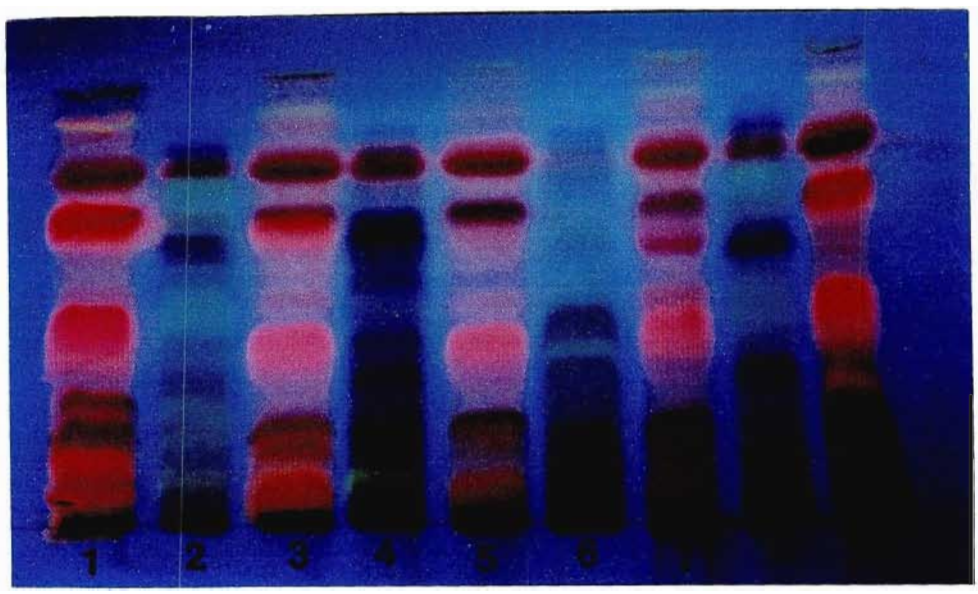


Figure 3.3.6 TLC plate of leaf and bark ethanolic extracts stained with potassium hydroxide reagent. Green flourescensing bands indicates coumarins. Lanes 1, 3, 5, 7 and 9 are the leaf extracts of *E. caffra*, *E. humeana*, *E. latissima*, *E. lysistemon* and *E. zeyheri*, respectively. Lanes 2, 4, 6 and 8 are the bark extracts of *E. caffra*, *E. humeana*, *E. latissima* and *E. lysistemon*, respectively.

Table 2.2.1 shows that very few coumarins have been isolated from the genus *Erythrina*. Figure 3.3.6 clearly shows that there are quite a number of coumarins present in this genus. The leaves do not appear to contain any coumarins but the bark seems to have quite a few. *E. caffra* and *E. lysistemon* appear to contain more coumarins than any of the other species. They also seem to have similar banding patterns with respect to these compounds.

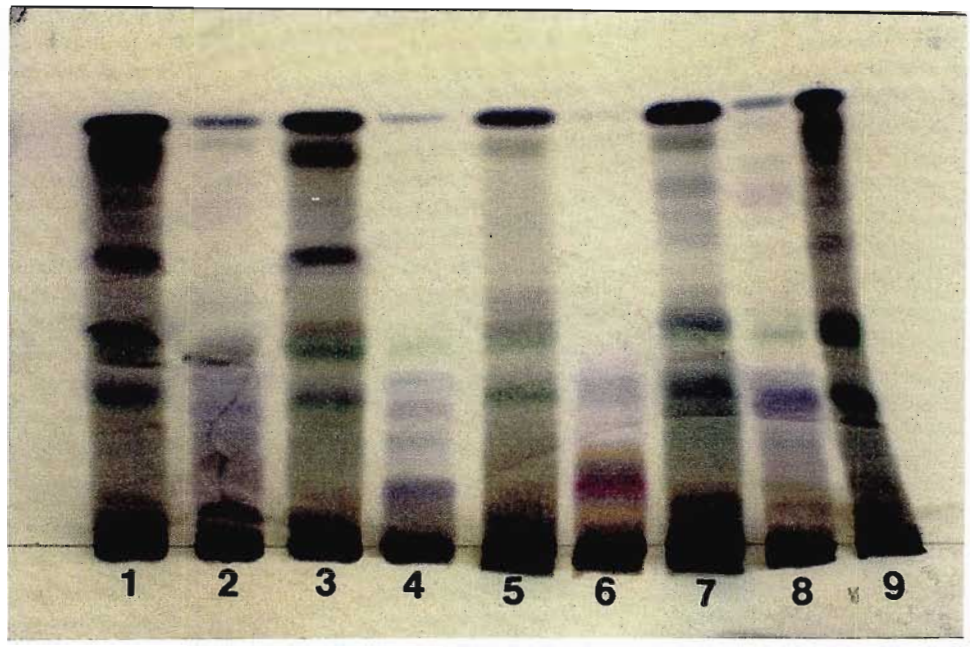


Figure 3.3.7 TLC plate of the leaf and bark ethanolic extracts stained with anisaldehyde-sulphuric acid reagent. Blue violet colour indicates the presence of triterpenes. Lanes 1, 3, 5, 7 and 9 are the leaf extracts of *E. caffra*, *E. humeana*, *E. latissima*, *E. lysistemon* and *E. zeyheri*, respectively. Lanes 2, 4, 6 and 8 are the bark extracts of *E. caffra*, *E. humeana*, *E. latissima* and *E. lysistemon*, respectively.

From the TLC analysis it is clear that very few triterpenes are found in the five species tested. The bark seems to contain more triterpenes than the leaves. The bark of *E. latissima* clearly shows a very distinct presence of triterpenes unlike the bark extracts of the other species. From Table 2.2.1 it is clear that very few triterpenes have been isolated from this genus.

3.4 CONCLUSIONS

Table 2.2.1 revealed that most of the species from the genus *Erythrina* are chemically rich plants. It also showed that not many compounds have been isolated from the

species indigenous to South Africa. This investigation did however, confirm the presence of alkaloids, flavonoids, coumarins and triterpenes in five of the local species.

This investigation also indicated a great similarity between *E. caffra* and *E. lysistemon*. The chemical profiles of these two species appear to be similar in most cases (Figures 3.3.1, 3.3.2, 3.3.3 and 3.3.6). There also appears to be similarities amongst the other species in the profiles of both the leaf and bark extracts. The profile of the bark extract of *E. latissima* appears to differ considerably from the other profiles of the bark extracts.

The presence of the various compounds raises a few questions; " What is the purpose of all these compounds in the plant?". A more important question is; "How can the presence of these compounds benefit humans and animals in the cycle of life?" and lastly, "May they serve as a useful chemo-taxonomic tool in the future?".

CHAPTER FOUR

SCREENING FOR ANTI-INFLAMMATORY ACTIVITY

4.1 INTRODUCTION

A. Inflammation

Inflammation is a mechanism by which protective immune processes can be localized at a region where infection occurs (VILLEE, SOLOMON, MARTIN, MARTIN, BERG & DAVIS, 1989). When a pathogen invades mammalian tissue it triggers an array of responses. Blood vessels in the affected area dilate and increases the flow of blood to the infected area. This increased blood flow causes the skin to look red and to feel warm. Capillaries in the inflamed area become more permeable causing more fluid to leave the circulation and to enter the tissues. As the amount of interstitial fluid increases swelling (edema) occurs. This swelling causes pain (ZURIER, 1982). Figure 4.1.1 is a diagrammatic representation of the inflammation process.

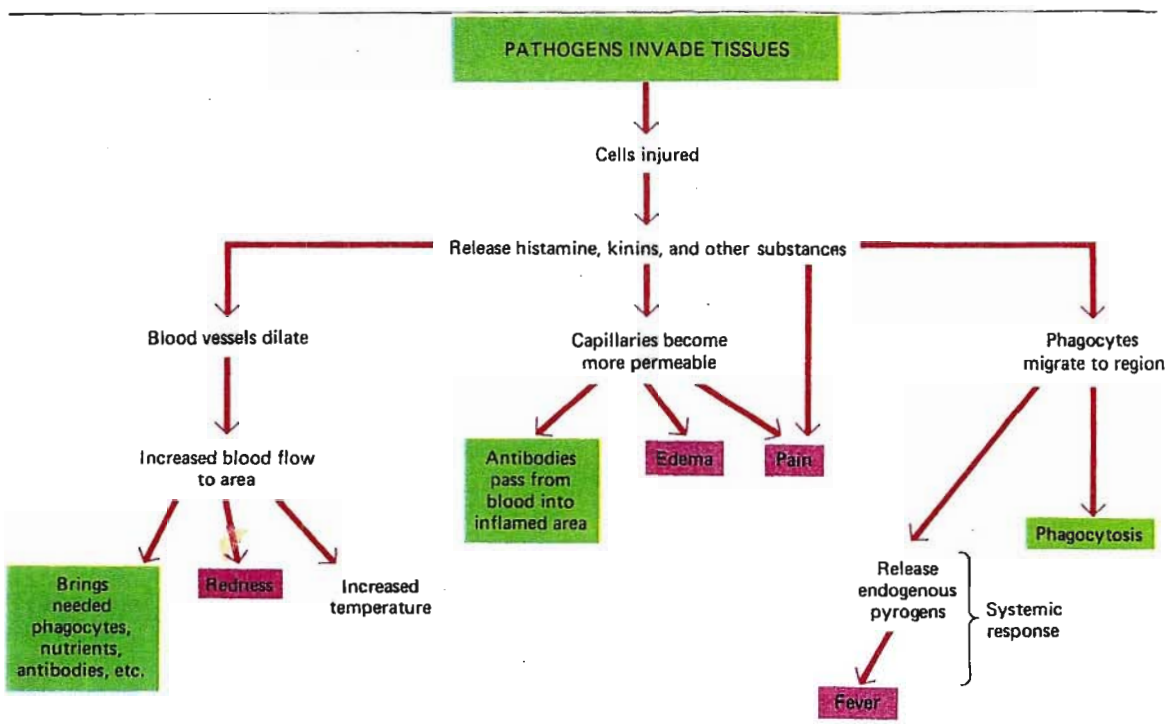


Figure 4.1.1 Diagrammatic representation of the inflammation process (VILLEE, SOLOMON, MARTIN, MARTIN, BERG & DAVIS, 1989)

B. The biochemical basis of inflammation

Inflammation is induced by prostaglandins. Prostaglandins are the products of the metabolism of C_{20} polyenoic fatty acids that contains the five-membered carbon ring. Figure 4.1.2 below shows how prostaglandins are synthesized.

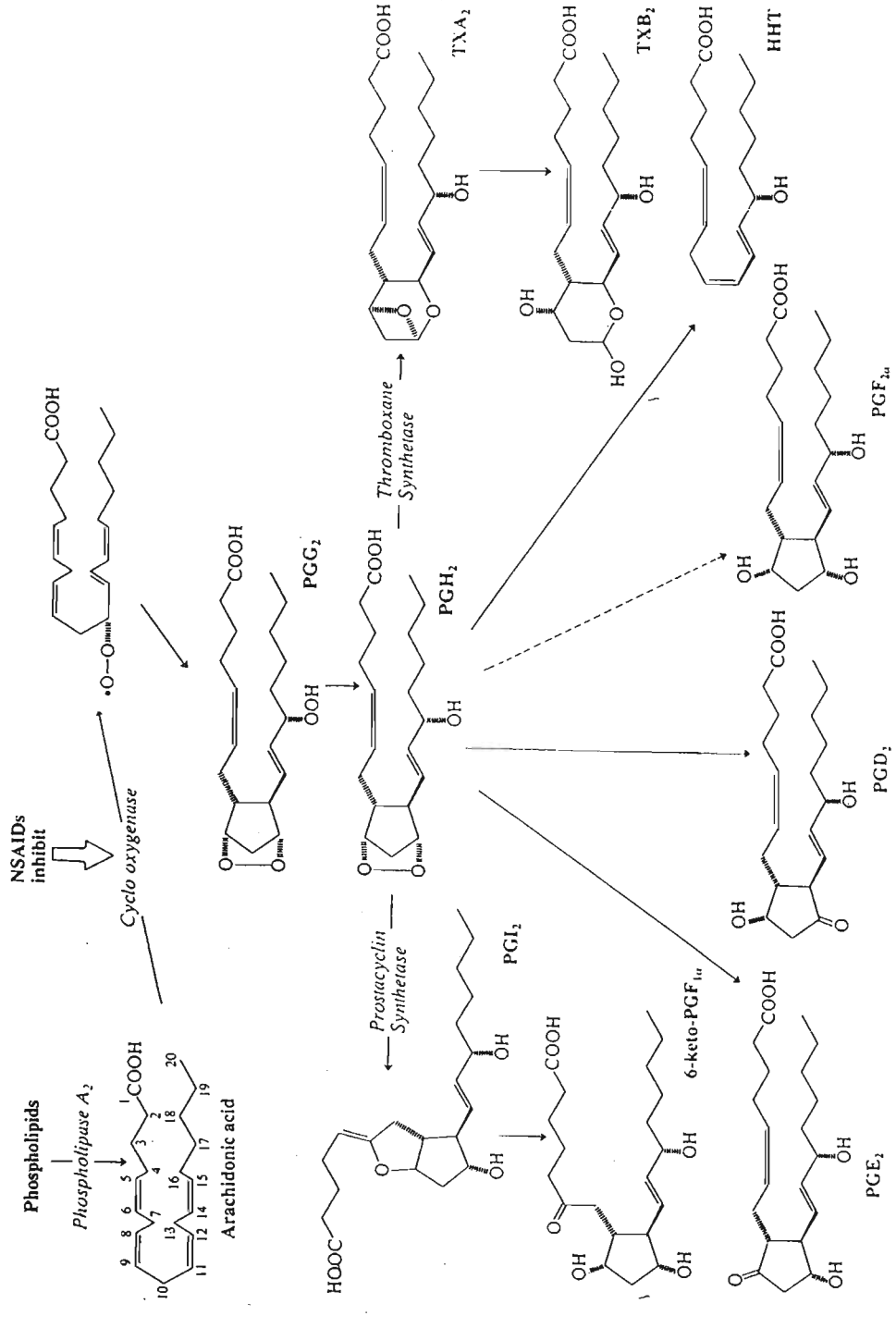


Figure 4.1.2 Diagrammatic representation of prostaglandin synthesis (RANG & DALE, 1987)

Prostaglandins are synthesized via endoperoxides from arachidonic acid and other essential fatty acids. The arachidonic acid is converted to endoperoxide PGG_2 and via three different pathways to prostacyclin (PGI_2); prostaglandins (PGE_2, PGF_2 and PGD_2; and thromboxones (A_2 and B_2) (GANONG, 1987).

C. The cyclooxygenase enzymes

Cyclooxygenase is the enzyme involved in the first step of prostanoid synthesis. Two isoenzymes exist in the mammalian body; the constitutive COX-1 and the inducible COX-2. These isoenzymes are encoded by two different genes but they produce prostaglandins along the same pathway as can be seen in Figure 4.1.3 (TAKETO, 1998).

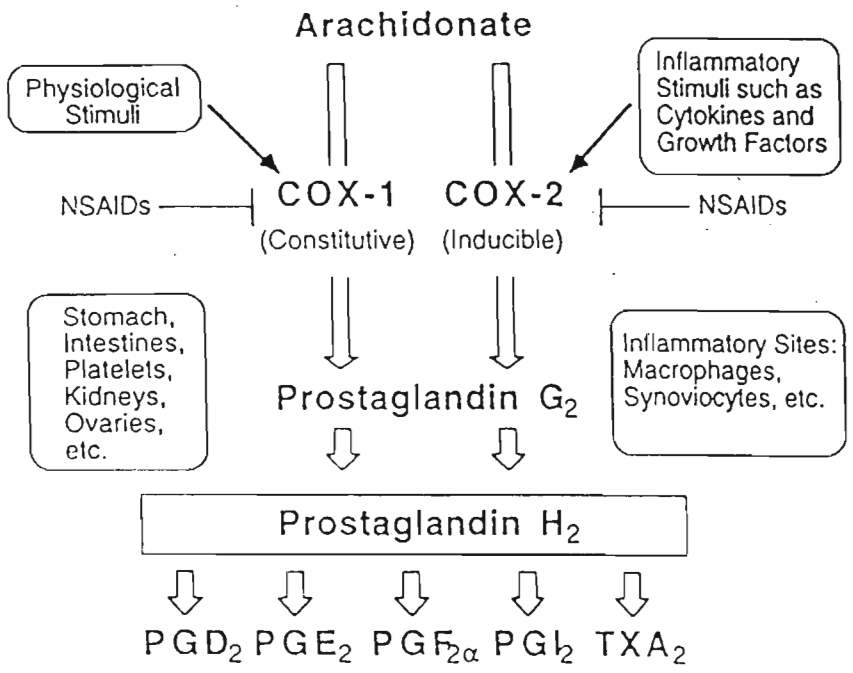


Figure 4.1.3 Diagrammatic representation of the COX-1 and COX-2 pathways (TAKETO, 1998)

The two isoenzymes regulates prostaglandin synthesis. COX-1 is expressed constitutively and ubiquitously and COX-2 is produced in response to certain stimuli (TAKETO, 1998). COX-1 produces prostaglandins that are involved in "house-keeping" activities such as regulating vascular homeostasis, stomach function, renal water and sodium resorption. COX-2 is not produced in resting tissues and prostaglandins

produced by this enzyme is probably involved only secondarily in prolonged physiological reactions (SMITH & DEWITT, 1995).

D. Primary structure of the cyclooxygenase isoenzymes

Figure 4.1.4 below compares the deduced amino acid sequences of human COX-1 and COX-2.

```
2     MLARALLLCAVL-------------------ALSHTANPCCSHPCQNRGVCMSVGFDQYKCD
1     MSR-SLLLRFLLLLLLL-PPLP-VLLADPGAPTPVNPCCYYPCQHQGICVRFGLDRYQCD
                10        20        30        40        50

2     CTRTGFYGENCSTPEFLTRIKFLLKPTPNTVHYILTHFKGFWNVVNNIPFLRNAIMSYVL
1     CTRTGYSGPNCTIPGLWTWLRNSLRPSPSFTHFLLTHGRWFWEFVNAT-FIREMLMLLVL
       60        70        80        90        100       110

2     TSRSHLIDSPPTYNADYGYKSWEAFSNLSYYTRALPPVPDDCPTPLGVKGKKQLPDSNEI
1     TVRSNLIPSPPTYNSAHDYISWESFSNVSYYTRILPSVPKDCPTPMGTKGKKQLPDAQLL
         120       130       140       150       160       170

2     VGKLLLRRKFIPDPQGSNMMFAFFAQHFTHQFFKTDHKRGPAFTNGLGHGVDLNHIYGET
1     ARRFLLRRKFIPDPQGTNLMFAFFAQHFTHQFFKTSGKMGPGFTKALGHGVDLGHIYGDN
         180       190       200       210       220       230

2     LARQRKIRLFKDGKMKYQIIDGEMYPPTVKDTQAEMIYPPQVPEHLRFAVGQEVFGLVPG
1     LERQYQLRLFKDGKLKYQVLDGEMYPPSVEEAPVLMHYPRGIPPQSQMAVGQEVFGLLPG
         240       250       260       270       280       290

2     LMMYATIWLREHNRVCDVLKQEHPEWGDEQLFQTSRLILIGETIKIVIDDYVQHLSGYHF
1     LMLYATLWLREHNRVCDLLKAEHPTWGDEQLFQTTRLILIGETIKIVIEEYVQQLSGYFL
         300       310       320       330       340       350

2     KLKFDPELLFNKQFQYQNRIAAEFNTLYHWHPLLPDTFQINDQKYNYQQFIYNNSILLEH
1     QLKFDPELLFGVQFQYRNRIATEFNHLYHWHPLMPDSFKVGSQEYSYEQFLFNTSMLVDY
         360       370       380       390       400       410

2     GITQFVESFTRQIAGRVAGGRNVPPAVQKVSQASIDQSRQMKYQSFNEYRKRFMLKPYES
1     GVEALVDAFSRQIAGRIGGGRNMDHHILHVAVDVIRESREMRLQPFNEYRKRFGMKPYTS
         420       430       440       450       460       470

2     FEELTGEKEMSAELEALYGDIDAVELYPALLVEKPRPDAIFGETMVEVGAPFSLKGLMGN
1     FQELVGEKEMAAELEELYGDIDALEFYPGLLLEKCHPNSIFGESMIEIGAPFSLKGLLGN
         480       490       500       510       520       530

2     VICSPAYWKPSTFGGEVGFQIINTASIQSLICNNVKGCPFTSFSVPDPELIKTVTINASS
1     PICSPEYWKPSTFGGEVGFNIVKTATLKKLVCLNTKTCPYVSFRVPDASQDDGPAVE---
         540       550       560       570       580       590

2     SRSGLDDINPTVLLKERSTEL
1     ---------------RPSTEL
                        599
```

Figure 4.1.4 Stucture of COX-1 and COX-2 (SMITH & DEWITT, 1995)

COX-1 differs significantly from COX-2 at positions before amino acid residue 30. Processed COX-1 has 576 amino acids and an *N*-terminal sequence ADPGA. Mature COX-2 has an *N*-terminal sequence ANPCC corresponding to the cleavage of a 17-amino acid signal peptide. COX-2 contains a cassette of 18 amino acids near the *C*-terminus which is absent in COX-1. Although the sequences near the *N*-termini are relatively unique, the remaining core sequences of COX-1 and COX-2 are 75% identical (SMITH & DEWITT, 1995).

E. Inhibitors of the cyclooxygenase enzymes

The main inhibitors of the cyclooxygenase enzymes and hence inflammation are the non-steroidal anti-inflammatory drugs (NSAID's). These drugs have three major types of effects; (RANG & DALE, 1987) :

i. **Anti-inflammatory effect** - Modifying the inflammatory reaction;

ii. **Analgesic effect** - Reducing pain; and

iii. **Antipyretic effect** - Lowering a raised temperature.

Depending on the dose these NSAID's can have a number of adverse effects. They can cause gastric upset, delay the birth process and damage the kidneys (TAKETO, 1998). Based on their binding kinetics with the COX enzymes these NSAID's can be grouped into three classes (SMITH & DEWITT, 1995) :

i. **Class I NSAID's** - These are simple, competitive inhibitors that compete reversibly with arachidonate for binding to the cyclooxygenase site.

ii. **Class II NSAID's** - These are time-dependant, competitive inhibitors of cyclooxygenase activity. These agents bind rapidly and reversibly in a first phase to form an enzyme-inhibitor complex. If retained for a sufficient time in the active site it causes a conformational change in the protein. Once bound in this tighter form, these time-dependant NSAID's only slowly dissociate from the cyclooxygense active site.

iii. **Class III NSAID's** - These are non-competitive inhibitors that bind irreversibly to the cyclooxygenase active site. Table 4.1.1 contains examples of drugs of all three the classes.

Table 4.1.1 Three classes of NSAID's based on their inhibition kinetics (TAKETO,1998).

Class I NSAID's	Ibuprofen
	Mefenamic acid
	Piroxicam
	Sulindac sulfide (active site of sulindac)
	Naproxen
	6-MNA (active site of nabumetone)
	Phenylbutazone
	Flufenamic
	BL-2365
Class II NSAID's	Indomethacin
	Fluribiprofen
	Meclofenamic acid
	Diclofenac
	BL-2338
	BF 389
	DuP 697
	NS - 398
Class III NSAID's	Aspirin
	Valeryl salicylate

4.2 MATERIALS AND METHODS

4.2.1 Microsomal isolation

A. Isolation of the cyclooxygenase enzyme

Eight seminal vesicles (approximately 4 cm in diameter) were removed from 1-2 year old rams at Abakor, the Cato Ridge Abattoir. The vesicles were transported on ice and then frozen in liquid nitrogen and stored at -70 ^{0}C overnight. The frozen vesicles were cut into small 1x1 cm pieces on a glass petri dish on ice. Sixty five grams of the cut vesicles was placed into 150 ml 0.1 M potassium phosphate - EDTA buffer, pH 7.4. The vesicles were ultraturaxed at medium speed for 10 min. The homogenate was then

placed into an ultrasound bath for 5 min. The homogenate was centrifuged at 4 000 g (Beckman Avanti J-25 I) for fifteen minutes. The supernatant was decanted and centrifuged at 17 000 g (Beckman Avanti J-25 I).The microsomes were isolated by ultracentrifugation at 100 000 g (Beckman L7 -5S) for one hour. The microsomal pellet was resuspended in 0.1 M potassium phosphate buffer, pH 7.8.

B. Determining the enzyme concentration

The enzyme concentration was determined using the Bio-Rad Protein Assay Kit. The standard procedure was followed except that 20 µl of each standard or sample solution were diluted with 1 ml of the dye reagent. The absorbance was read at 595 nm. Once the concentration had been determined 100 µl aliquots of the enzyme were pipetted into Eppendorf tubes and frozen at -70 ^{0}C.

C. Optimizing conditions for the enzyme for use in the prostaglandin biosynthesis bioassay

i. Enzyme concentration

A range of concentrations between 0 and 1% (0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1.0) were used in the the prostaglandin biosynthesis bioassay.

ii. Incubation time

To determine the correct incubation time for the reaction between the substrate (arachidonic acid) and the enzyme a range of incubation times between 0 and 12 min (0, 3, 5, 7, 9 and 12) were used.

4.2.2 Screening for anti-inflammatory activity

A. Plant material

Plant material (bark and mature leaves) of *E. caffra, E. humeana, E. latissima, E. lysistemon* and *E. zeyheri* was collected from the Botany Departmental Garden at the University of Natal, Pietermaritzburg during February 1998. A voucher specimen of each of the five species that was investigated, i.e. *E. caffra (Pillay2*NU*), E. humeana (Pillay* 5NU*), E. latissima (Pillay4*NU*), E. lysistemon (Pillay1*NU*)* and *E. zeyheri (Pillay3*NU*)* was deposited in the Herbarium of the University of Natal, Pietermaritzburg.

B. Extraction of plant material

The plant material was dried in an oven at 50 ^{0}C and stored at room temperature in brown paper bags until further use. One gram of finely ground plant material was extracted individually with 10 ml ethyl acetate, ethanol or water, respectively, in an ultrasound bath for 30 min. The extracts were filtered and dried. The residues were resuspended in ethanol to a final concentration of 20 mg ml^{-1} for the cyclooxygenase bioassay.

C. Anti-inflammatory activity

Inhibition of prostaglandin biosynthesis by the plant extracts was investigated by using the cyclooxygenase bioassay. The bioassay was performed according to the method of WHITE & GLASSMAN (1974) as modified by JÄGER, HUTCHINGS & VAN STADEN (1996).

Cyclooxygenase enzyme solution (4 µg protein prepared from sheep seminal vesicles) and co-factor solution (0.3 mg ml^{-1} of L-adrenalin and reduced glutathione in 0.1 M Tris buffer, pH 8.2) was mixed in a 1:5 ratio and incubated on ice for 15 min. Sixty µl of enzyme / co-factor solution was added to 17.5 µl water and 2.5 µl ethanolic plant extract or 2.5 µl indomethacin standard solution (20 mM). Twenty µl of ^{14}C- arachidonic acid was added to the aqueous samples and incubated at 37 ^{0}C for 8 min. The reaction was terminated by adding 10 µl 2N HCl. A background sample (17.5 µl water and 2.5µl ethanol) was kept in an ice bucket. After incubation 4 µl of a 2 mg ml^{-1} carrier solution of unlabelled prostaglandins (PGE_2 : PGF_2 1:1; v/v) was added.

The ^{14}C-labelled prostaglandins synthesised during the assay were separated from unmetabolized ^{14}C-arachidonic acid by column chromatography. Silica gel in eluent 1 (hexane : dioxan : acetic acid 350 : 150 :1; v/v/v) was packed in Pasteur pipettes to a height of 3 cm. One ml of eluent 1 was added to each of the assay mixtures and this mixture was applied to separate columns. The arachidonic acid was eluted with 4 ml of eluent 1. The prostaglandins were subsequently eluted with 3 ml eluent 2 (ethyl acetate : methanol 85 : 15; v/v) into scintillation vials. After mixing with scintillation solution, the radioactivity was counted using a Beckman LS 6000LL scintillation counter. Two different concentrations of the plant extract (2 and 20 mg ml^{-1} residue) were assayed, with double determinations for each extract. The percentage inhibition of the test

solutions was obtained by analysing the amount of radioactivity in these solutions relative to that present in the solvent blank.

The percentage cyclooxygenase inhibition was calculated using the following formula:

$$1 - \frac{(\text{dpm of sample} - \text{dpm of background})}{(\text{dpm of blank} - \text{dpm of background})} \times 100$$

D. Thin layer chromatography

Ten µl of the crude ethyl acetate and ethanol plant extracts (20 mg ml^{-1}) of *E. caffra, E. lysistemon* and *E. latissima* were spotted onto 20x20 cm glass 0.25 mm Merck silica gel TLC plates. The plates were developed in hexane : ethyl acetate 2:1 (v/v). Spots that were visible under normal light , ultraviolet light; (254 and 366 nm) and after staining with 4N H_2SO_4 were demarcated. Each spot from a similar non-stained plate was scraped off separately and the compound eluted with ethanol. The samples were filtered through 0.45 µm Millipore filters and dried. The residues were resuspended to 20 mg ml^{-1} and 2 mg ml^{-1} , respectively and tested for anti-inflammatory activity using the cyclooxygenase bioassay.

4.3 RESULTS AND DISCUSSION

4.3.1 Microsomal isolation

A. Determining the concentration of the enzyme stock solution

The 10 fold diluted enzyme solution had an absorption of 0.55 at 595 nm. This translates to a concentration of 0.83 mg ml^{-1} for the diluted solution and 8.3 mg ml^{-1} for the enzyme solution.

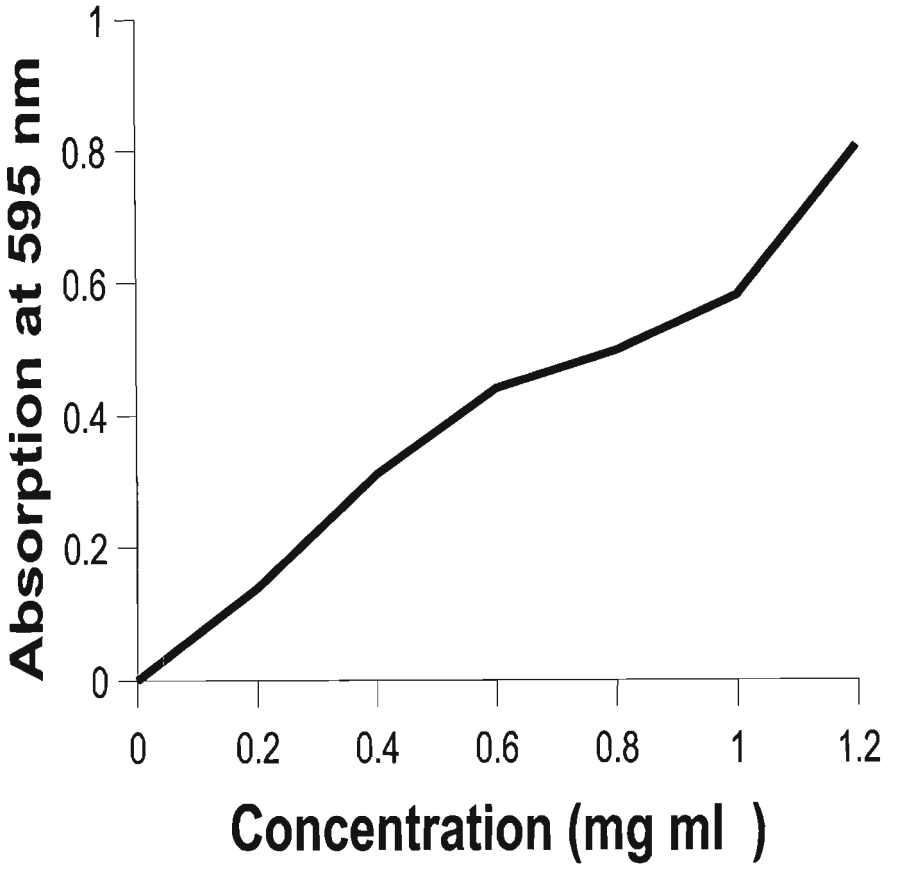


Figure 4.3.1.1 Standard curve for the Bio-Rad Protein Assay

B. Optimizing conditions for the enzyme for use in the prostaglandin biosynthesis bioassay

i. Enzyme concentration

To ensure that the enzyme is saturated during incubation time, normally no more than 30% of the substrate should be converted. On the other hand it is desirable to have as high counts as possible with the amount of added radioisotope. In the present assay 6 000 to 8 000 dpm was close to 30% of the substrate converted.

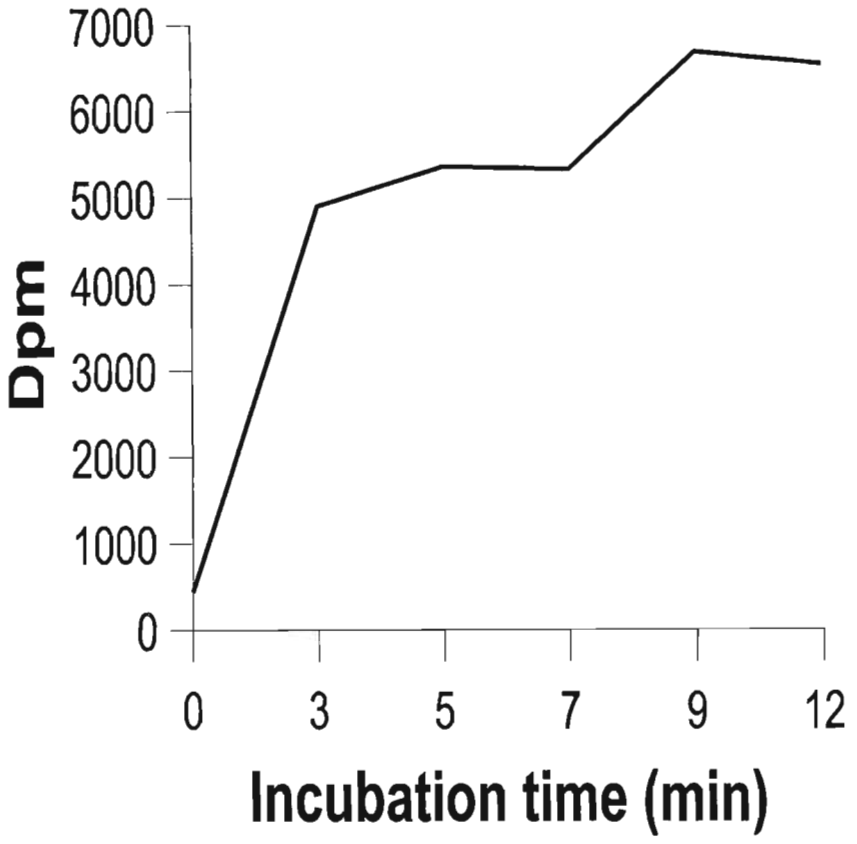


Figure 4.3.1.2 Curve to determine the optimal enzyme concentration to use in the cyclooxygenase bioassay.

From Figure 4.3.1.2 this conversion is obtained with enzyme concentrations of 0.5 mg ml^{-1} to 0.6 mg ml^{-1}. An enzyme concentration of 0.55 mg ml^{-1} was therefore used in the bioassay.

ii. Incubation time

The incubation time curve showed an increase in product formation during the first 10 min, although not at a linear rate. An incubation time of 10 min was therefore used.

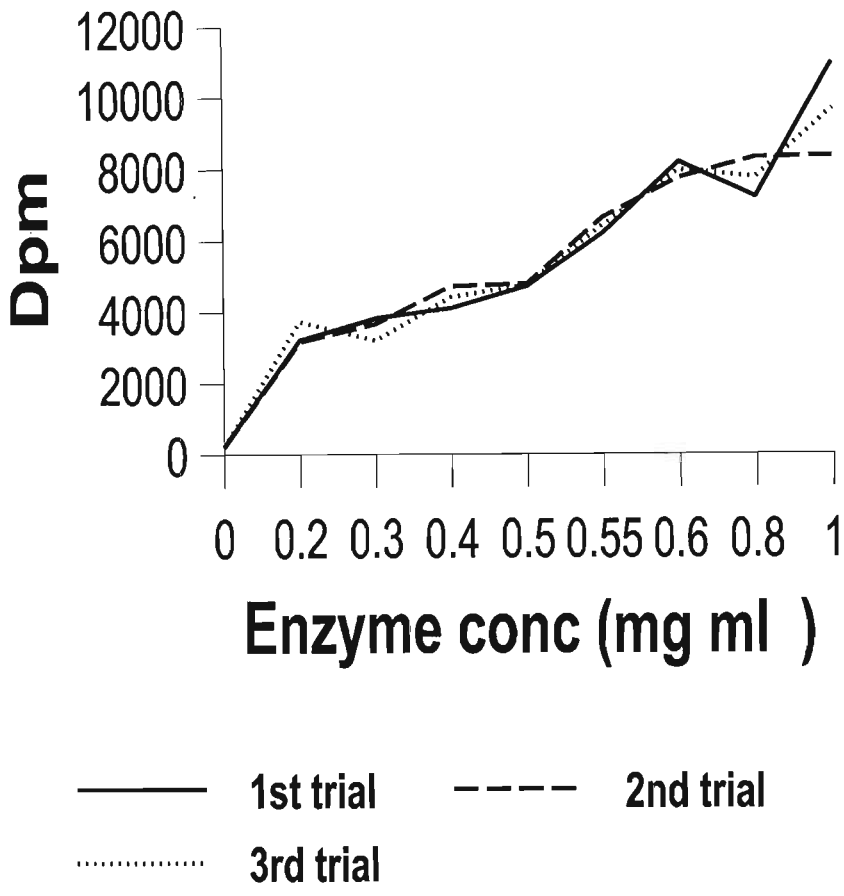


Figure 4.3.1.3 Curve to determine the optimal incubation time for the reaction mixture.

4.3.2 Screening for anti-inflammatory activity

A. Anti-inflammatory activity

The results of the screening for inhibitors of cyclooxygenase are presented in Figure 4.3.2.1. In general plant material extracted with ethyl acetate and ethanol showed higher inhibition of cyclooxygenase than did material extracted with water. The water extracts showed no activity except for the leaf extract of *E. zeyheri* but the inhibition was very low.

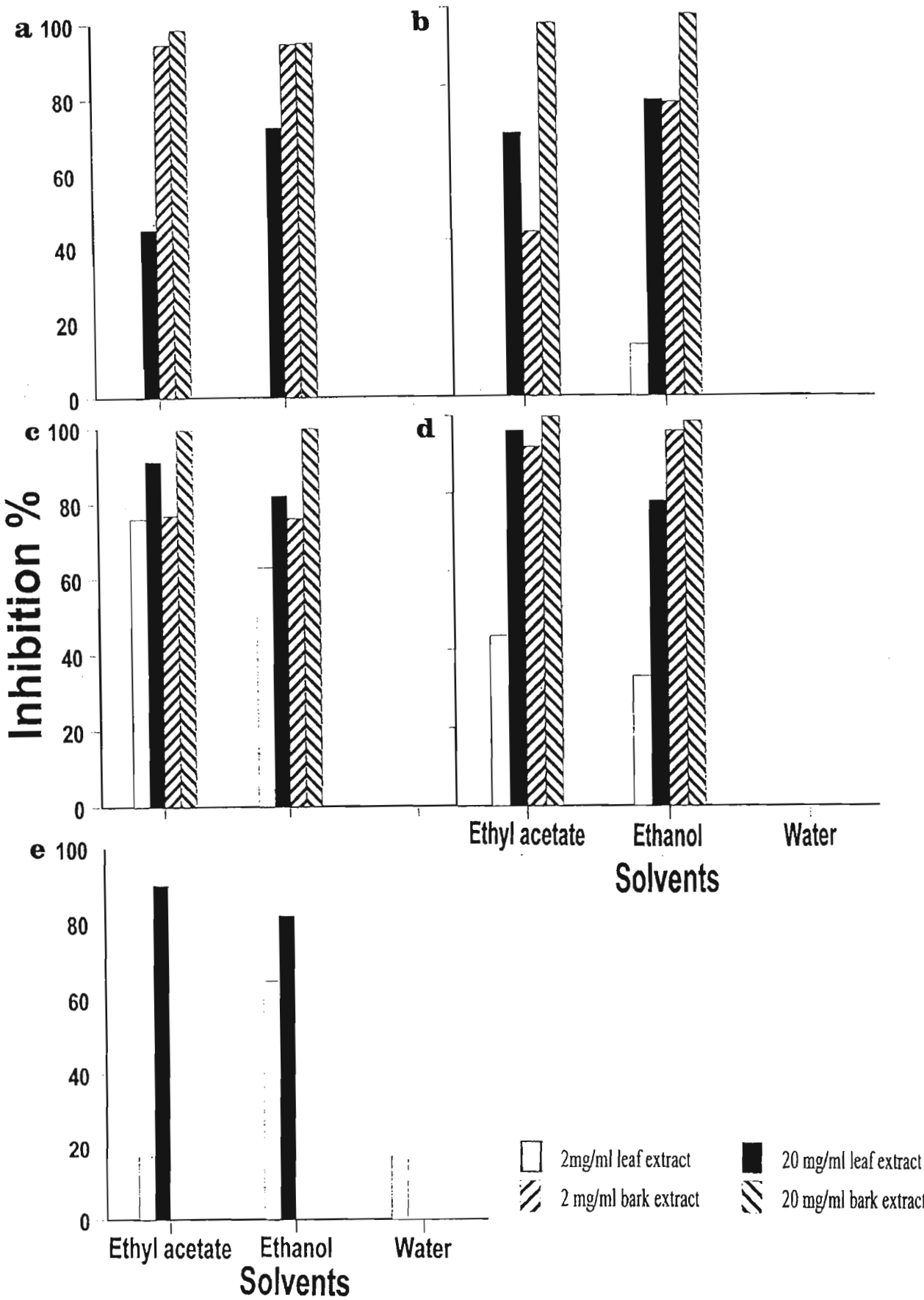


Figure 4.3.2.1 Percentage cyclooxygenase inhibition displayed by a) *E. caffra*, b) *E. humeana*, c) *E. latissima*, d) *E. lysistemon* and e) *E. zeyheri*. 20 mM indomethacin displayed an inhibition percentage of 86%.

The bark extracts generally yielded higher inhibition of cyclooxygenase than the leaves. This is in agreement with the literature, as it is reported that traditional healers use the bark more frequently than the leaves to cure certain ailments (Table 2.2.2). COX (1995) reported that Hedge and Patel isolated anti-inflammatory compounds, two flavanoids; 4'-hydroxy-3',5'-diprenyl and 3,9-dihydroxy-2,10-diprenyl pterocarp-6a-ene and a novel isoflavanone; 4'-hydroxy-3',5',6-triprenyl, from the bark of *Erythrina variegata*.

E. caffra and *E. lysistemon* displayed considerable inhibition of cyclooxygenase. The ethyl acetate and ethanol extracts of these two species displayed an inhibition of more than 90% for both the 2 mg ml^{-1} and 20 mg ml^{-1} extracts. This considerable activity at 2 mg ml^{-1} is an indication of the presence of very potent compound(s) in the bark of these two species. *E. caffra* and *E. lysistemon* are the two species most frequently used by traditional healers (Table 2.2.2).

C. Thin layer chromatography

From the chromatographic results in Figure 4.3.2.2 it is evident that ethyl acetate and ethanol extract similar compounds. It can also be seen that *E. caffra* and *E. lysistemon* have very similar chromatographic profiles; indicating that it might be the same compound(s) in the two species conferring anti-inflammatory activity. The similar chromatographic profiles is yet another similarity between the two species. *E. caffra* and *E. lysistemon* was once classified as the same species. Their morphological traits are almost identical with the only visible difference being in the flowers. In Figure 4.3.2.2 it is also clear that there are a large number of compounds in *E. caffra* and *E. lysistemon* with cyclooxygenase inhibitory activity greater than the indomethacin standard.

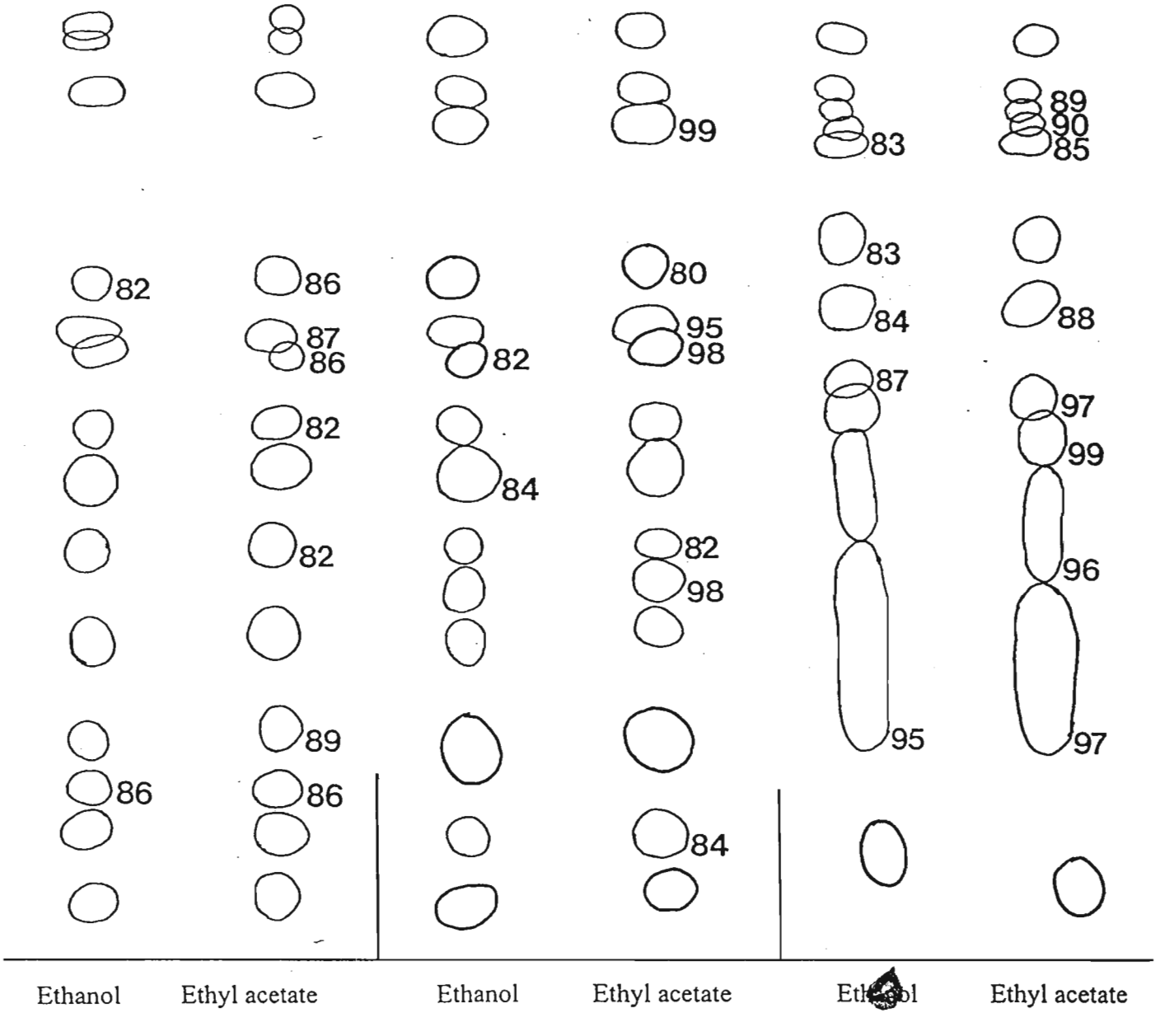


Figure 4.3.2.2 TLC separation of ethyl acetate and ethanol bark extracts of *E. caffra, E. lysistemon* and *E. latissima.* Spots with cyclooxygenase inhibition percentage greater than 80% are indicated in the diagram.

4.4 CONCLUSIONS

The bark is more active than the leaves for most of the species tested, especially the bark of *E. caffra* and *E. lysistemon*. The water extracts generally yielded no activity. The chemical profiles of *E. caffra* and *E. lysistemon* are very similar as indicated in Chapter three. From the chromatograms it appears that ethanol and ethyl acetate extract the same types of compounds. Figure 4.3.3.2 shows that there are a number of compounds in all three of the species that display significant cyclooxygenase inhibitory activity. Compound isolation is therefore a viable venture.

CHAPTER FIVE
ATTEMPTED ISOLATION OF ANTI-INFLAMMATORY COMPOUNDS FROM THE BARK OF *E. lysistemon*

5.1 INTRODUCTION

Although the discovery of new drugs from plants is a taxing process in terms of time and finance it is a worthwhile long term venture to undertake. From Table 1.3.2 it is clear that plants are a good source of drugs.

Asprin was one of the first drugs discovered and developed by scientists when the medicinal properties of plants were being explored (COX & BALCK, 1994). Aspirin is an anti-inflammatory drug and is one of the most widely used anti-inflammatory drugs in the world. Even though it has its beneficial effects in the alleviation of inflammation it is not without its negative effects. One of the greatest negative effects is that it erodes the lining of the stomach. Most of the other anti-inflammatory drugs have the same effect on the lining of the stomach. The search for new anti-inflammatory drugs that would not have these negative effects is without doubt a reason to search for new anti-inflammatory drugs.

Based on the results of the screening for anti-inflammatory activity of the five *Erythrina* species it seemed logical to pursue the isolation of anti-inflammatory compounds from the bark of *E. lysistemon*.

5.2 MATERIALS AND METHODS

In an attempt to isolate active compounds bioassay guided fractionation was carried out. The flow diagram below is a summary of the methods used in the purification process.

Bulk extraction with hexane

⇓

Vacuum liquid chromatography

⇓

Chromatotron

⇓

Vacuum liquid chromatography

⇓

Thin layer chromatography

⇓

High pressure liquid chromatography

Figure 5.2.1 Flow diagram of methods used in compound isolation

A. Bulk extraction

One kg of dried bark of *E. lysistemon* was cut into 1x1 cm pieces. These pieces of bark were placed into a Soxhlet apparatus and extracted with 2.5 l of hexane for 30 hours.

B. Vacuum liquid chromatography

Three grams of the hexane extract was dried under vacuum and placed onto a Merck silica (150 g) vacuum column (30x6cm). Compounds were eluted with 400 ml hexane : ethyl acetate. A solvent gradient was used consisting of 100% : 0% to 0% : 100% (v/v) hexane : ethyl acetate, in 5% steps. Four hundred ml fractions were collected and dried under a vacuum. The residues were resuspended to 20 mg ml^{-1} and tested for cyclooxygenase inhibitory activity using the cyclooxygenase bioassay (Chapter 4 for protocol). Ten µl of each fraction was spotted onto a 20x10 cm plastic Merck silica gel TLC plate. The plate was developed in hexane : ethyl acetate

2 : 1 (v/v).

C. Chromatotron

The fraction that displayed the highest cyclooxygenase inhibitory activity was dried under vacuum. The residue was placed onto a 2 mm chromatotron plate. The plate was developed in a solvent gradient consisting of 100% : 0% to 70% :30% (v/v) hexane : ethyl acetate, in 10% steps. The total volume of the solvent mixture was 100 ml. The bands that were visible at 254 and 366 nm were collected as separate fractions. These fractions were dried under vacuum. The residues were resuspended to 20 mg ml^{-1} and tested for cyclooxygenase inhibitory activity using the cyclooxgenase bioassay. Ten µl of each fraction was spotted onto a 20x10 cm plastic Merck silica gel TLC plate. The plate was developed in hexane : ethyl acetate 2 : 1 (v/v).

D. Vacuum liquid chromatography using a small column

The fraction that displayed the highest cyclooxygenase inhibitory activity was dried under a vacuum. This residue was placed onto a Merck silica (25 g) vacuum column(17x3cm). Compounds were eluted with 100 ml hexane : ethyl acetate. A solvent gradient was used consisting of 100% : 0% to 0% : 100% (v/v) hexane : ethyl acetate, in 5% steps. One hundred ml fractions were collected and dried under a vacuum. The residues were resuspended to 4 mg ml^{-1} and tested for cyclooxygenase inhibitory activity using the cyclooxygenase bioassay. Ten µl of each fraction was spotted onto a 20x10 cm plastic Merck silica gel TLC plate. The plate was developed in hexane : ethyl acetate 2 : 1 (v/v).

E. Thin layer chromatography

One hundred miligrams of the fraction that displayed the highest cyclooxygenase inhibitory activity was dried under vacuum. The residue was resuspended in a minimum amount of ethanol and streaked onto five 20x20 cm glass 0.25 mm Merck silica gel TLC plates. These plates were run in toluene : ethyl acetate 3 : 1 (v/v). The plates were viewed under normal and ultraviolet light, (254 and 366 nm). The visible bands were demarcated and each band was scraped off separately. The compound(s) in each band was eluted with ethanol. The samples were filtered through celite to remove the silica.

Each sample was dried and the residue resuspended to 4 mg ml^{-1}. These samples were tested for cyclooxygenase inhibitory activity using the cyclooxygenase bioassay.

F. High pressure liquid chromatography

Samples that displayed the highest cyclooxygenase inhibitory activity were purified further on a Varian (5000) HPLC. These samples were run separately on a C-18 semi-preparative Phenomenex column (250x10 mm) in 80% : 20% (v/v) methanol : water. The different compounds present in the samples were detected with a uv 3000HR detector at 200 nm. The flow rate was 2.5 ml min^{-1} and fractions were collected at 1 min intervals. These fractions were then tested for cyclooxygenase inhibitory activity. The area or peak that displayed the highest cyclooxygenase inhibitory activity was collected.

G. Testing the lack of activity in samples I and J

Fractions I and J were run separately on a C-18 semi-prepreparative Phenomenex column (250x10 mm) in 80% : 20% (v/v) and 100% : 0% (v/v) methanol : water. Unlike before fractions were not collected. The entire samples were collected. These samples were dried and the residues were resuspended to 4 mg ml^{-1}. They were then tested for cyclooxygenase inhibitory activity.

5.3 RESULTS AND DISCUSSION

A. Vacuum liquid chromatography

From Table 5.3.1 it is evident that fractions D, E, F, G, I, J and K displayed considerable cyclooxygenase inhibitory activity at 20 mg ml^{-1}.

Table 5.3.1 Cyclooxygenase inhibitory activity displayed by fractions from the vacuum column.

Fractions	Mass of residue (mg)	% Inhibition at 20 mg ml^{-1}	% Inhibition at 2 mg ml^{-1}
A	410	32	
B	620	25	
C	1090	80	
D	540	99	93
E	270	100	88
F	290	100	80
G	160	100	79
H	160	89	
I	60	100	79
J	20	100	76
K	10	99	64
L	60	60	
M	30	89	
Indomethacin	-	96	88

At 2 mg ml^{-1} fraction D displayed the highest cyclooxygenase inhibitory activity. The activity displayed by fraction D was higher than that of indomethacin indicating the presence of a very potent compound in this fraction. The compounds in fraction D (Figure 5.3.1) also seem to separate out quite easily in a hexane : ethyl acetate solvent system. Fraction D was therefore used for the next step of the purification process.

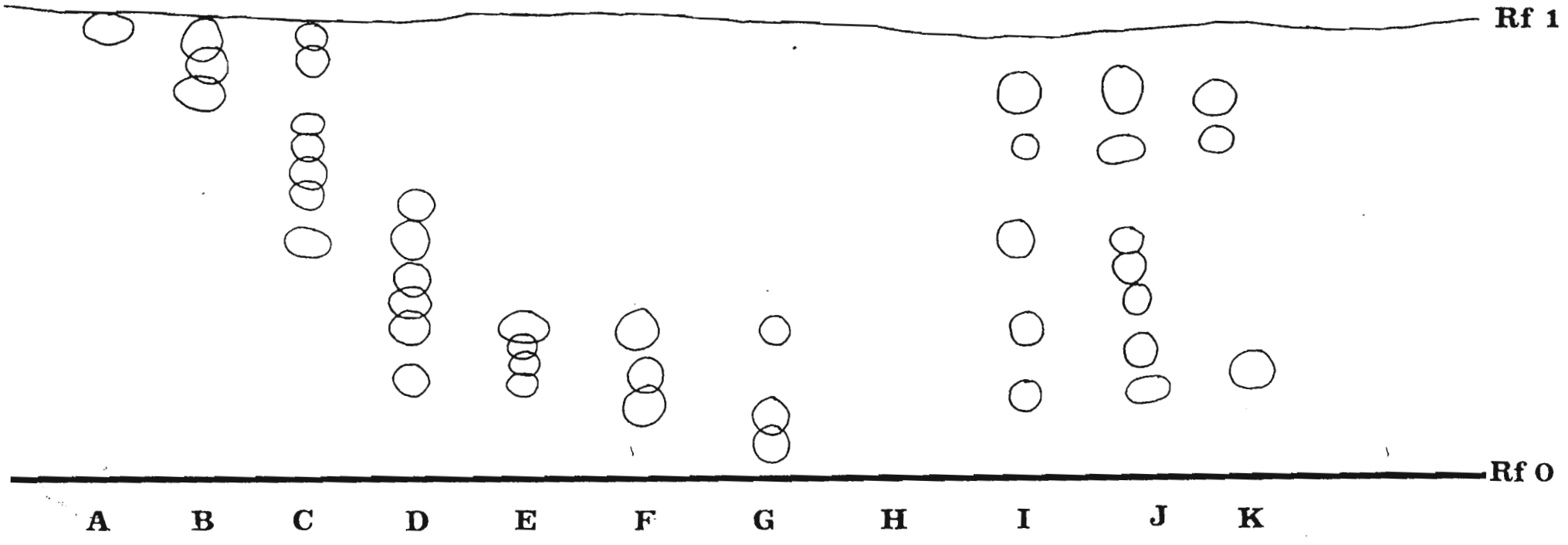


Figure 5.3.1 TLC plate of fractions from the vacuum column

Figure 5.3.1 shows that each of the fractions that was eluted from the column contained different compounds. There are, however, some overlaps where the same spot would appear in different fractions. These spots would probably be found in varying concentrations in the different fractions.

B. Chromatotron

From Table 5.3.2 it is clear that fractions B and C displayed the highest cyclooxygenase inhibitory activity of all the fractions that were collected and tested, however, fractions A, B, C and D contain the same compounds (Figure 5.3.2). The lower inhibitory activity of fractions A and D could possibly be because of a decrease in concentration of the active compound. These four fractions were therefore combined and purified further on a small vacuum column.

Table 5.3.2 Cyclooxygenase inhibitory activity displayed by fractions from the chromatotron.

Fractions	Mass of residue (mg)	% Inhibition at 20 mg ml^{-1}
A	20	80
B	67	95
C	126	95
D	42	82
E	89	67
F	160	83
G	120	70
Indomethacin	-	81

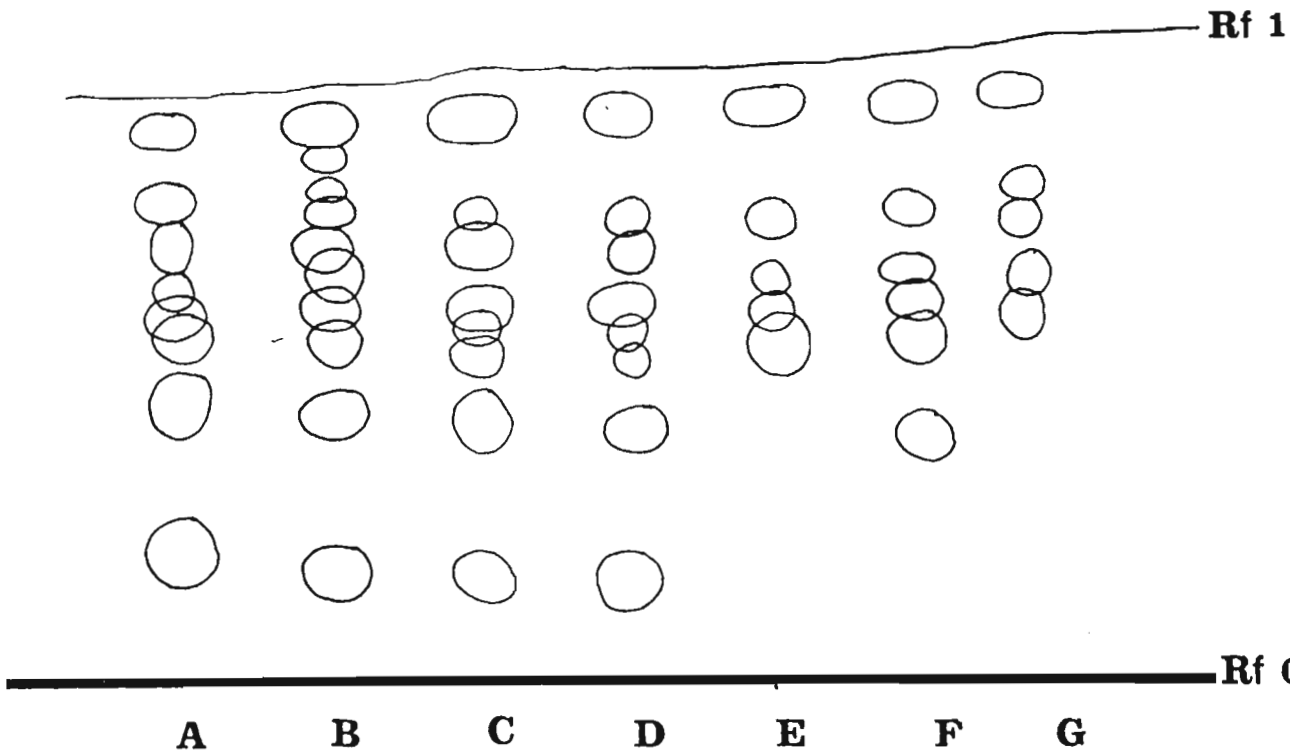


Figure 5.3.2 TLC plate of fractions from the chromatotron

C. Vacuum liquid chromatography using a small column

Fractions I, J, K, M, N, O and P all displayed a considerable amount of anti-inflammatory activity at 4 mg ml^{-1} and were therefore diluted to 0.25 mg ml^{-1} and tested for anti-inflammatory activity. A further dilution to 0.025 mg ml^{-1} revealed that fraction O was the most active.

Table 5.3.3 Cyclooxygenase inhibitory activity displayed by fractions from a small vacuum column.

Fractions	Mass of residue (mg)	% Inhibition at 4 mg ml^{-1}	% Inhibition at 0.25 mg ml^{-1}	% Inhibition at 0.025 mg ml^{-1}
A	190	13		
B	120	32		
C	20	7		
D	70	20		
E	80	23		
F	130	61		
G	40	88		
H	10	65		
I	90	92	88	
J	40	95	93	25
K	50	96	92	17
L	90	79		
M	20	98	97	27
N	380	93	69	
O	100	98	98	82
P	10	92	54	
Indomethacin	-	88	84	90

Fraction O was very active considering the percentage cyclooxygenase inhibitory activity of 82% at a concentration of 0.025 mg ml^{-1}. This is expected because as a sample becomes purer the more concentrated the active compound becomes. Fraction O, however consisted of at least 4 compounds as is seen on the thin layer chromatogram (Figure 6.3.3).

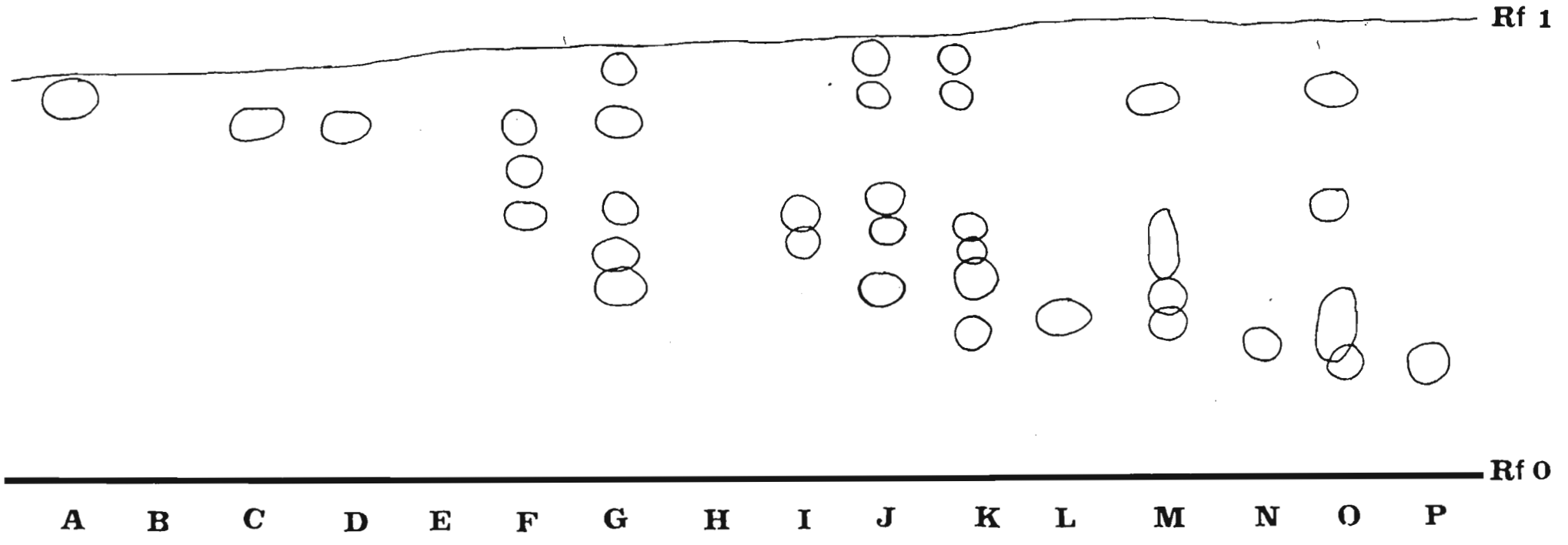


Figure 5.3.3 TLC plate of fractions from the small vacuum column

D. Thin layer chromatography

The compounds eluted from spots H, I and J displayed a high cyclooxygenase inhibitory activity. As the bands were overlapping there might have been some contamination and only one of the bands could represent the active compound.

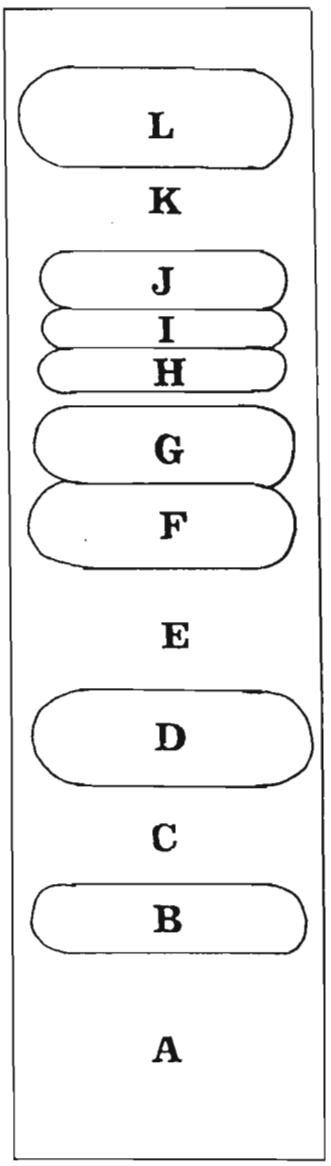


Figure 5.3.4 TLC plate of fraction O

Table 5.3.4 Cyclooxygenase inhibitory activity displayed by spots of fraction O.

Spot or space	Mass of residue (mg)	% Inhibition at 4 mg ml^{-1}
A	20	32
B	10	30
C	10	59
D	20	5
E	20	14
F	10	66
G	10	67
H	20	79
I	30	89
J	20	79
K	10	29
L	30	0
Indomethacin	-	80

E. High performance liquid chromatography

Cyclooxygenase inhibitory activity could only be detected at the peak labelled (a). This peak was collected and from Figure 5.3.6 it is clear that it is a pure compound. The noise at the baseline is probably very small quantities of other compounds. These compounds are, however, in low concentrations. The symmetry of the peak is an indication of its purity. However, once this peak was separated from the other compounds in the sample it had no cyclooxygenase inhibitory activity.

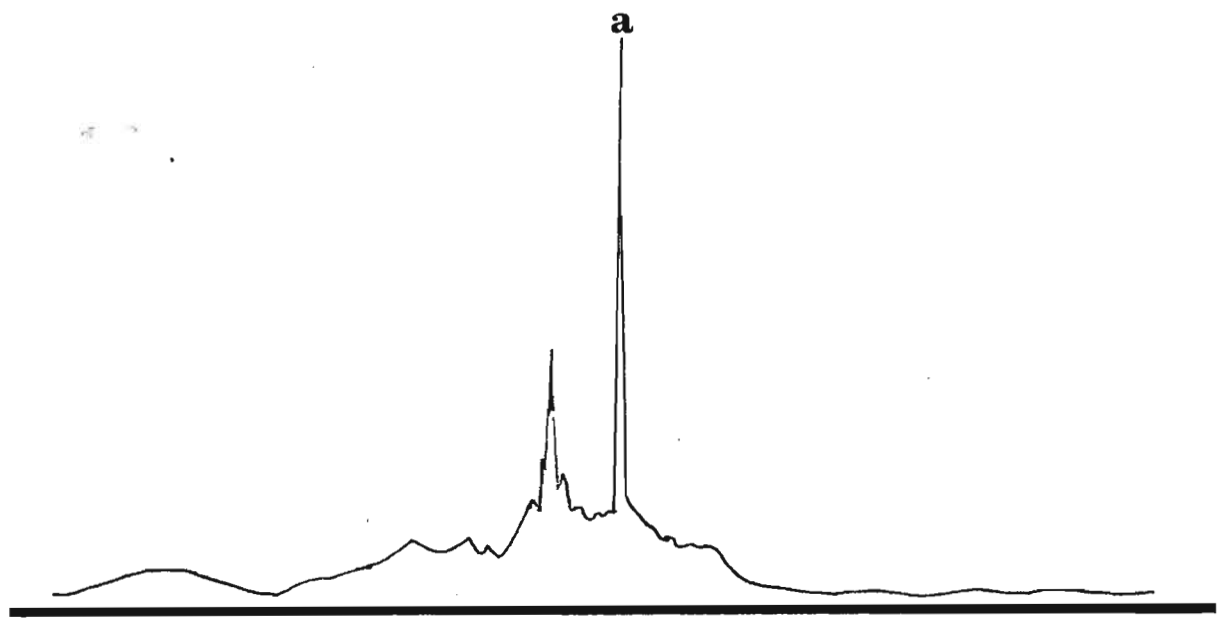


Figure 5.3.5 HPLC chromatogram of fraction H

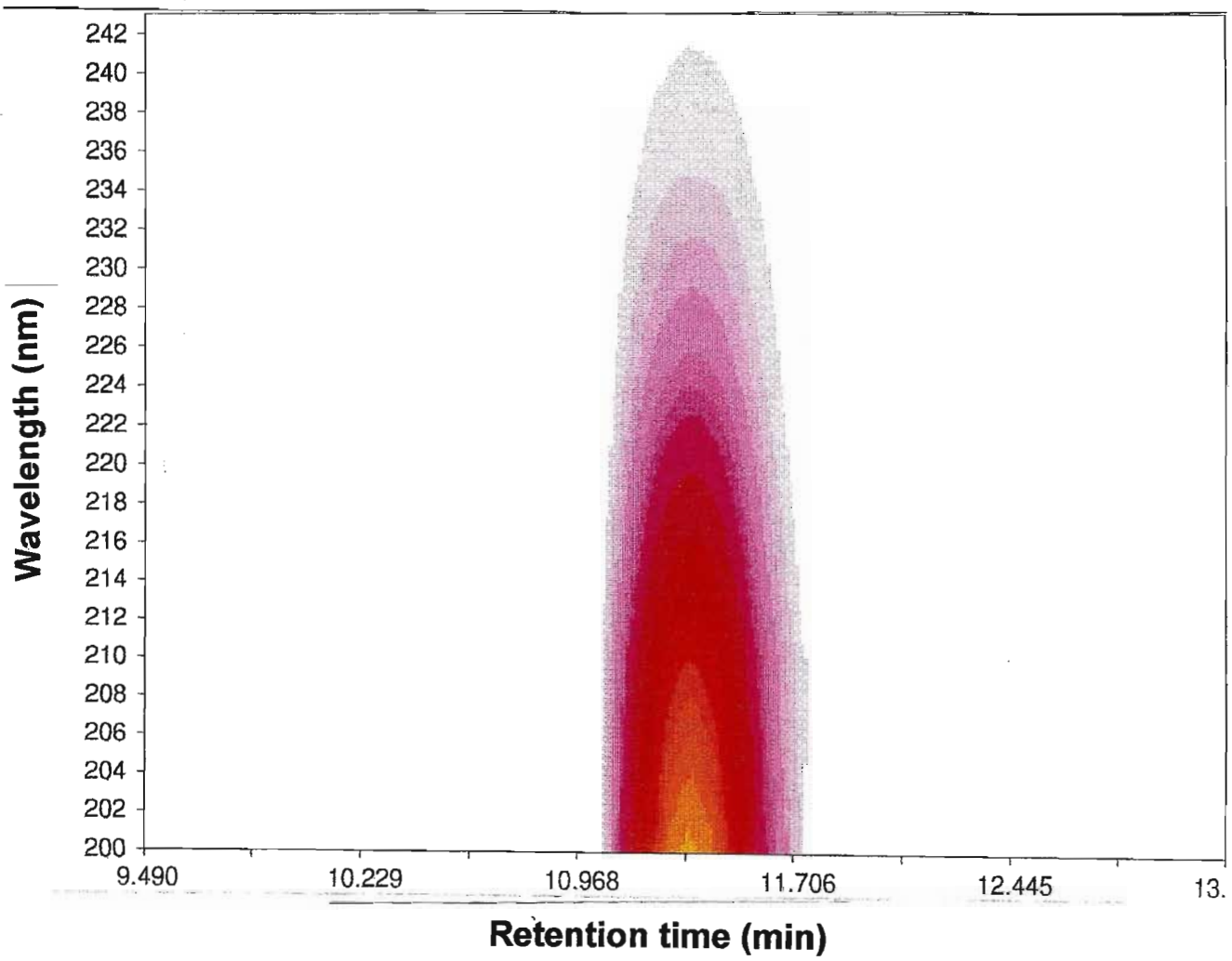


Figure 5.3.6 Ultraviolet spectrum of the active peak

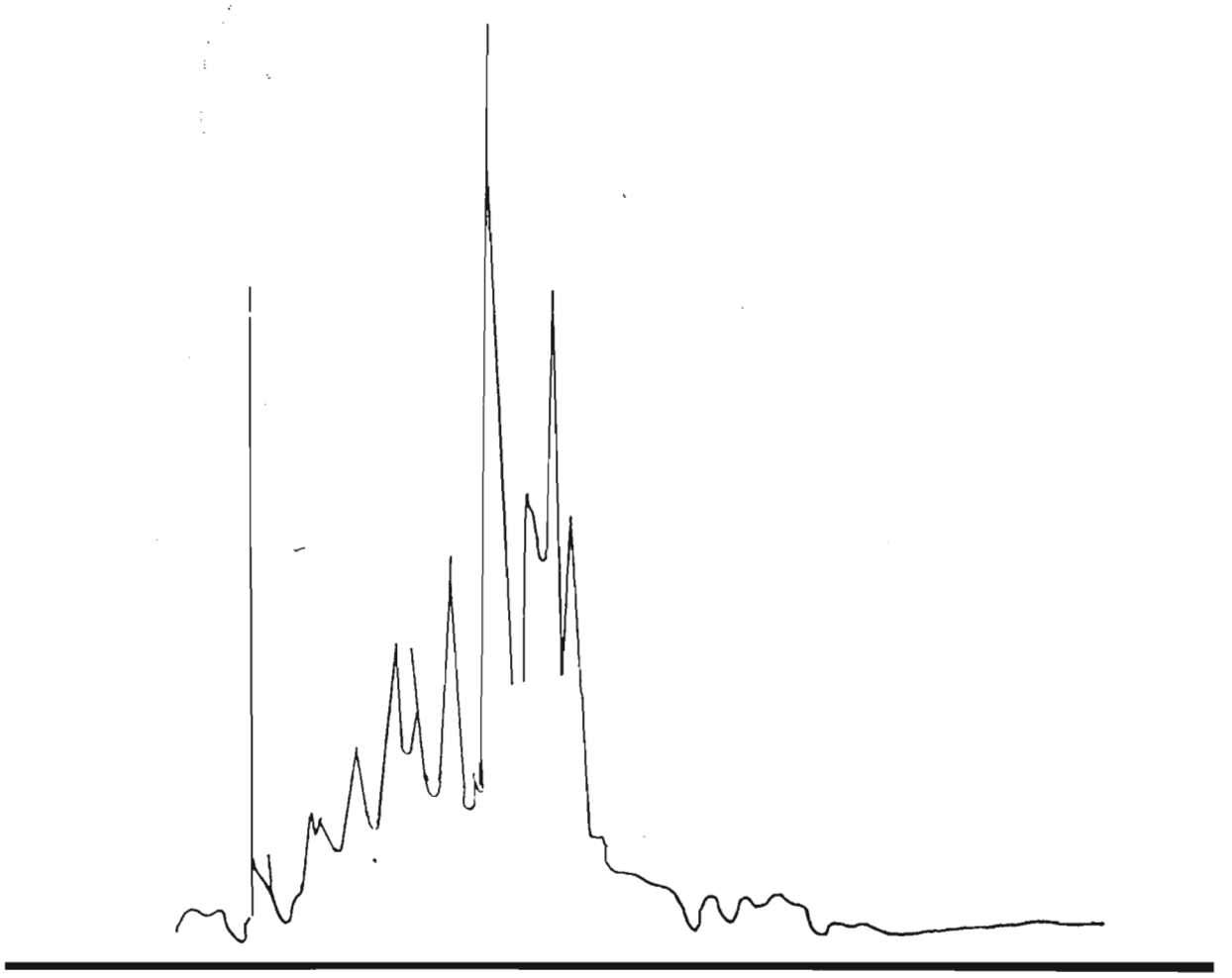


Figure 5.3.7 HPLC chromatogram of fraction I

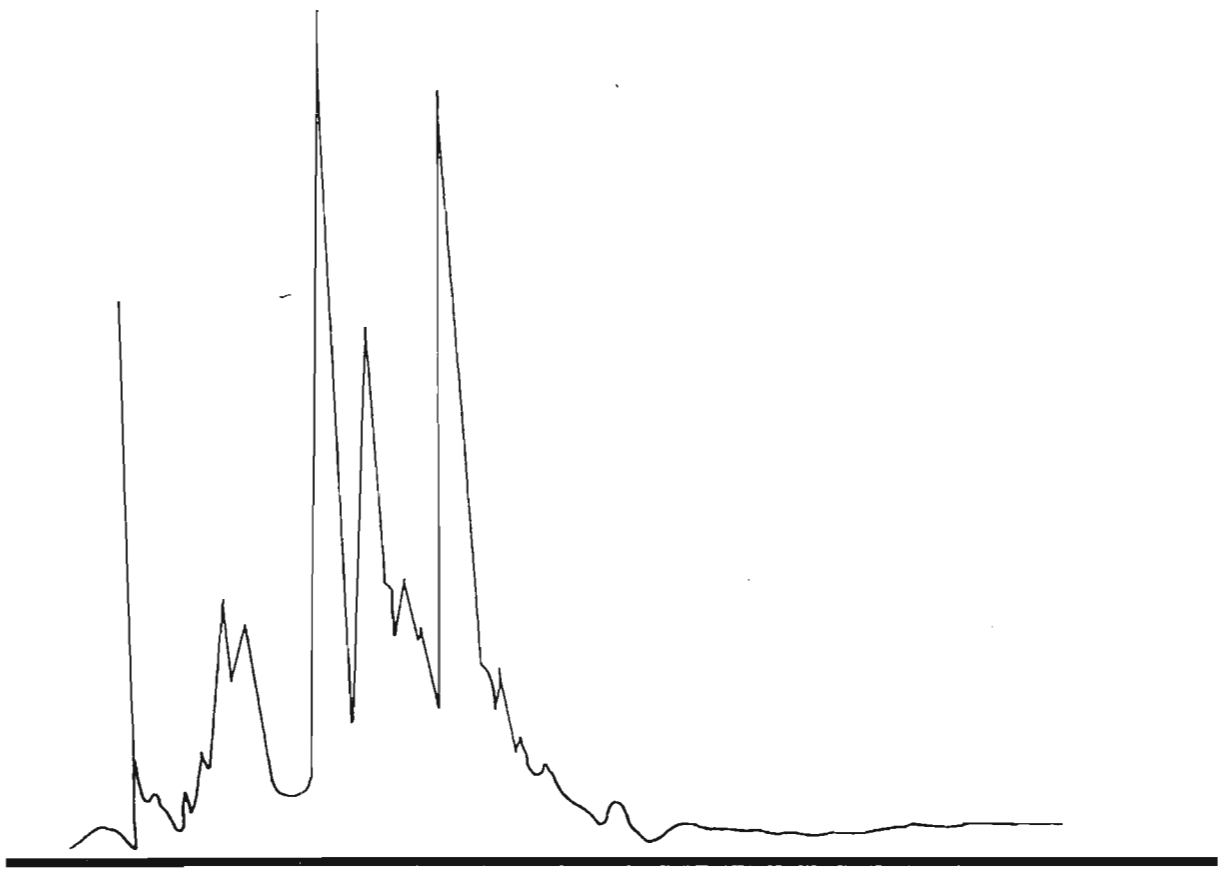


Figure 5.3.8 HPLC chromatogram of fraction J

No cyclooxygenase inhibitory activity could be detected in any of the fractions collected. The chromatograms of samples I and J show that there are a number of compounds in each of these fractions but once these compounds are separated the cyclooxygenase inhibitory activity disappears. The cyclooxygenase inhibitory activity that I and J displayed could have been as a result of contamination from the compound(s) in fraction H (Figure 5.3.4).

F. Testing the lack of activity in samples I and J

Samples I and J displayed no cyclooxygenase inhibitory activity for the samples that was run in 80% and 100% methanol. Peaks were not collected separately and therefore the inactivity in I and J could not be attributed to the concept of synergism;

where two or more molecules act together to confer biological activity. Samples I and J were collected as entire samples without separation of the different compounds found in them. The lack of activity could therefore be attributed to the molecules breaking down on the HPLC.

5.4 CONCLUSIONS

The isolation of an anti-inflammatory compound proved to be unsuccessful. Although the crude extract and some of the extracts obtained from the purification process displayed considerable activity, the pure compound that was obtained had no cyclooxygenase inhibitory activity. It was determined that the active compounds break down on the HPLC.

CHAPTER SIX

SCREENING FOR ANTI-BACTERIAL ACTIVITY

6.1 INTRODUCTION

Anton van Leeuwenhoek was the first to describe microscopic "streaks and threads". In 1850 Casmir Dawaine characterized these microscopic organisms as bacteria (ALCAMO, 1994). It was only as the twentieth century unfolded that scientists realized that we live in the center of a microbial universe.

Bacteria are prokaryotes and are amongst the most abundant organisms on earth. They have adapted to more different living conditions than any other group of organisms. They inhabit the air, soil and water and exist in enormous numbers on the surfaces of virtually all plants and animals. On all sides microscopic organisms surround us and make their presence felt for good or bad. The useful species outnumber the harmful species by far and are of such value to humans that we cannot live without them. The remaining species are agents of disease and death.

A. Disease causing bacteria

Bacteria have been involved in the great plagues of history and still are today. Bacteria found in the air, soil and water all cause a wide array of diseases in humans some of which are curable and some not. Table 6.1.1 shows some of these diseases.

Table 6.1.1 Airborne, waterborne, foodborne and soilborne bacterial diseases

(ALCAMO, 1994)

	Disease	Causative agent	Description of agent
Airborne bacterial diseases	Strep throat	*Streptococcus pyogenes*	Gram positive, streptococcus
	Diphtheria	*Corynebacterium diphetria*	Gram positive, rod
	Whopping cough	*Bordella pertussis*	Gram negative, rod
	Meningococcal meningitis	*Neisseria meningtidis*	Gram negative, diplococcus
	Haemophilus meningitis	*Haemophilus influenzae b*	Gram negative, rod
	Tuberculosis	*Mycobacterium tuberculosis*	Acid-fast, rod
	Pneumococcal pneumonia	*Streptococcus pneumoniae*	Gram positive, diplococcus
	Klebsiella pneumonia	*Klebsiella pneumoniae*	Gram negative, rod
	Primary atypical pneumonia	*Mycoplasma pneumoniae*	Mycoplasma
	Serratia pneumonia	*Serratia marcescens*	Gram negative, rod
	Legionnaires' disease	*Legionella pneumophila*	Gram negative, rod
	Q fever	*Coxiella burnetti*	Rickettsia
	Psittacosis	*Chlamydia psittaci*	Chlamydia
	Chlamydial	*Chlamydia*	Chlamydia
Waterborne and foodborne diseases	Botulism	*Clostridium botulinum*	Gram positive, rod
	Staphylococcal food poisoning	*Staphylococcus aureus*	Gram positive, staphylococcus

	Disease	Causative agent	Description of agent
	Clostridial food poisoning	*Clostridium perfringens*	Gram positive, rod
	Salmonellosis	*Salmonella typhi*	Gram negative, rod
	Shigellosis	*Shigella*	Gram negative, rods
	Cholera	*Vibrio cholerae*	Gram negative, curved rod
	Infantile and traveller's diarrhea	*Escherichia coli*	Gram negative, rod
	Brucellosis	*Brucella species*	Gram negative, rod
	Campylobacteriosis	*Campylobacter jejuni*	Gram negative, rod
Soilborne bacterial diseases	Anthrax	*Bacillus anthracis*	Gram positive, rod
	Tetanus	*Clostridium tetani*	Gram positive, rod
	Gas gangrene	*Clostridium perfringens*	Gram positive, rod
	Leptospirosis	*Leptospira interrogans*	Spirochete
	Listeriosis	*Listeria monocytogenes*	Gram positive, rod
	Mellioidosis	*Pseudomonas pseudomallei*	Gram negative, rod

B. Anti-bacterial agents

More than 50 years have passed since the modern antibiotic era had the first clinical trial for penicillin in 1941. Medicine has since been transformed and the use of antibiotics has grown to enormous proportions.

The bulk of natural antibiotics have been isolated from soil microorganisms. It is estimated that about 5 000 to 10 000 natural antibiotics have been isolated and characterized and approximately 50 000 to 100 000 analogues have been synthesized but most of these compounds do not have any medicinal value (MITSCHER, DRAKE, GOLLAPUDI & OKWUTE, 1987).

The most important and frequently used antibiotics are shown in Table 6.1.2.

Table 6.1.2 Important antibiotics used worldwide (ALCAMO, 1994)

Antibiotic	Source
Sulfonamides	Synthetic
Isoniazid	Synthetic
Metronidazole	Synthetic
Chloroquine and primaquine	Synthetic
Fluoroquinolones	Synthetic
Penicillins	*Penicillium notatum* and *chrysogenum*
Cephalosporins	*Cephalosporium* species
Aminoglycosides	*Micromonospora* and *Streptomyces* species
Chloraphenicol	*Streptomyces venezuela*
Tetracyclines	*Streptomyces* species
Erythromycin	*Stretomyces erythraeus*
Vancomycin	*Streptomyces orientalis*
Rifampin	*Streptomyces mediterranei*
Clindamycin and lincomycin	*Streptomyces lincolenisis*
Bacitracin	*Bacillus subtilis*
Polymyxin	*Bacillus polymyxa*
Moxalactem	*Chromobacter violaceum*
Nystatin	*Streptomyces noursei*
Griseofulvin	*Penicillium janczewski*
Amphotericin B	*Streptomyces nodosus*

Most antibiotics act on the cell wall. They break down the cell wall of the bacterium cells, releasing all the cell contents. This inhibits the bacterial cells from reproducing and hence destroys the disease (ALCAMO, 1994).

6.2 MATERIALS AND METHODS

A. Extraction of plant material

Extraction was carried out as in Section 4.2.2 (B). The residues were resuspended in ethyl acetate, ethanol and water, respectively, to a final concentration of 100 mg ml^{-1}. The antibacterial bioassay was carried out using these extracts.

B. Anti-bacterial Activity

Inhibition of bacterial growth by plant extracts was investigated using the disc diffusion assay (RASOANAIVO & RATSIMAMANGA-UVERG, 1993). The following bacteria were used; *Staphylococcus aureus, Staphylococcus epidermis, Micrococcus luteus, Escherichia coli, Klebsiella pneumoniae* and *Pseudomonas aeruginosa.* All bacteria were maintained at 4 ^{0}C on nutrient agar plates.

Base plates were prepared by pouring 10 ml Mueller-Hinton (MH) agar (Biolab) into sterile petri-dishes. Molten MH agar held at 48 ^{0}C was inoculated with a broth culture (10^6 - 10^8 bacteria per ml) of the test organism and poured over the base plates. Ten µl of plant extract was applied per filter paper disc (6 mm diameter, Whatman no.3) resulting in a final concentration per disc of 1 mg plant extract. The discs were air dried and placed onto the top layer of the agar. Each extract was tested in quadruplicate (4 discs per plate) with a neomycin disc (200 µg or 500 µg) as a reference. The plates were incubated for 18-24 hr at room temperature. Antibacterial activity was expressed as a ratio of the inhibition zone produced by the plant extract and the inhibition zone produced by the neomycin reference.

C. Thin Layer Chromatography

Ten µl of the crude ethyl acetate and ethanol plant extracts (20 mg ml^{-1}) of *E. caffra, E. lysistemon* and *E. latissima* were spotted onto a 20x20 cm glass 0.25 mm Merck silica gel TLC plate. The plate was developed in hexane : ethyl acetate 2:1 (v/v). Spots that were visible under normal and ultraviolet light, (254 and 366 nm) were demarcated. The spots were tested for anti-bacterial activity using the bioautographic bioassay.

D. Bioautographic bioassay

The bioautographic bioassay was performed according to a method by SLUSARENKO, LONGLAND & WHITEHEAD (1989). An overnight culture of *Staphylococcus aureus* was pelleted by centrifugation at 3000 g (Beckman Avanti J-25 I). The pellet was resuspended in 2 ml of the supernatant. This bacterial suspension was added to 25 ml of molten Mueller Hinton agar (Biolab) supplemented with 1% (v/v) glycerol and 0,1% (w/v) 2,3,5-triphenyltetrazolium chloride (Sigma). This was poured over a TLC plate containing crude ethyl acetate and ethanolic extracts of *E. caffra, E. latissima* and *E. lysistemon.* After the medium had solidified the overlayed TLC plate was placed in a box lined with moist tissue paper and incubated overnight at 37 ^{0}C.

4.3 RESULTS AND DISCUSSION

A. Anti-bacterial activity

The results of the screening for anti-bacterial activity are presented in Table 6.3.1. In general the leaves displayed very little activity; with no activity in the ethyl acetate and ethanol extracts for all the species. The water extracts of *E. caffra, E. humeana, E. latissima* and *E. zeyheri* displayed some activity. The bark was more active than the leaves; displaying activity in all three solvent extracts.

TABLE 6.3.1 Anti-bacterial activity of indigenous *Erythrina* species

Species	Plant part	Solvent used for extraction	Bacterial strains used						
			S.a.	S.e.	B.s.	M.l.	E.c.	K.p.	P.a.
E. caffra	Leaf	Ethyl acetate	0	0	0	0	0	0	0
		Ethanol	0	0	0	0	0	0	0
		Water	0	0.88	0.44	0.61	0	0	0.63
	Bark	Ethyl acetate	0.73	0.94	0	0.53	0	0	0.7
		Ethanol	0.83	0.59	0	0.69	0	0	0
		Water	0	0.79	0	0.45	0	0	0.62
E. humeana	Leaf	Ethyl acetate	0	0	0	0	0	0	0
		Ethanol	0	0	0	0	0	0	0
		Water	0	0.7	0	0.62	0	0	0.61
	Bark	Ethyl acetate	0	0	0.36	0.79	0	0	0
		Ethanol	0.89	0	0	0	0.63	0	0
		Water	0	0	0	0.7	0	0	0
E. latissima	Leaf	Ethyl acetate	0	0	0	0	0	0	0
		Ethanol	0	0	0	0	0	0	0
		Water	0.77	0	0	0	0.6	0	0

Species	Plant part	Solvent used for extraction	Bacterial strains used						
			S.a.	S.e.	B.s.	M.l.	E.c.	K.p.	P.a.
	Bark	Ethyl acetate	0.88	0	0.31	0.52	0	0	0
		Ethanol	0.85	0	0.52	0.55	0	0	0
		Water	0	0	0	0.61	0	0	0
E.lysistemon	Leaf	Ethyl acetate	0	0	0	0	0	0	0
		Ethanol	0	0	0	0	0	0	0
		Ethanol	0	0	0	0	0	0	0
	Bark	Ethyl acetate	0.88	0	0.38	0.65	0	0	0
		Ethanol	0.68	0	0.37	0.7	0	0	0
		Water	0.83	0	0.45	0.65	0	0	0
E. zeyheri	Leaf	Ethyl acetate	0	0	0	0	0	0	0
		Ethanol	0	0	0	0	0	0	0
		Water	0.88	0	0	0	0	0	0

Bacteria : S.a., *Staphylococcus aureus*, S.e., *Staphylococcus epidermis*, B.a., *Bacillus subtilis*, M.l., *Micrococcus luteus*, E.c., *Escherichia coli*, K.p., *Klebsiella pneumonia*, P.a., *Pseudomonas aeruginosa*.

The antibacterial activity is expressed as a ratio of the inhibition zone of the extract (1 mg ml^{-1}) to the inhibition zone of the reference (neomycin 200-500 mg ml^{-1})

Activity was mainly detected against the Gram positive bacteria. This is to be expected as the growth of Gram negative bacteria is more difficult to inhibit. *P. aeruginosa* was the only Gram negative bacterium whose growth was inhibited with more than one extract. It was very interesting that the water extract of *E. latissima* inhibited the growth of *E. coli*. Although there was some inhibition of the Gram negative bacteria, the inhibitory activity displayed by the extracts was very low. Considerable inhibitory activity was displayed against Gram positive *S. aureus* and *S. epidermis.*

Hexane extracts from *E. sigmoidea* was found to have anti-bacterial activity against *S. aureus*. Erythrabyssin II was isolated from this species and found to have anti-bacterial activity against *S. aureus* and anti-fungal activity against *Aspergillus fumigatus* and *Cryptococcus neoformans* (NKENGFACK,VUOFFO, VARDAMIDES & FOMUM, 1994). Sigmoidin A and B, isolated from *E. sigmoidea* was found to have activity against *S. aureus* and *M. luteus* (BIYITI, PESANDO & PUISEUX-DAO, 1988). Eriothrin B and erybraedins A and C, isolated from *E. eriotricha* showed anti-bacterial activity against *S. aureus* (NKENGFACK, VARDAMIDES, FOMUM & MEYER, 1995).

In a recent report it was shown that bidwillon B and auriculatin, isolated from *E. x bidwilli* had anti-bacterial activity against *Fusobacterium nucleatum* and *Prevotella intermedia* (IINUMA, TANAKA, MIZUNO, YAMAMOTO, KOBAYASHI & YONEMORI, 1992).

A report by MITSCHER, DRAKE, GOLLAPUDI & OKWUTE (1987) stated that the bark of *E. berteroana*, *E. crista-galli* and *E. variegata* displayed anti-bacterial activity against *S. aureus* and *M. smegmatis*. They also found that the roots of *E. costaricans, E. fusca, E. indica* and *E. mildbraedii* displayed anti-bacterial activity against the same bacterial strains.

KOUAM, NKENGFACK & FOMUM (1991) reported that *n*-octacosanyl-4-hydroxy-3-methoxycinnamate, 6,3-diprenylgenistein and auriculatin from *E. senegalensis, E. excelsa* and *E. eriotricha* displayed activity against *S. aureus.* They also reported that these compounds displayed antirhymic effects as cardiovascular agents and anti-fungal activity against *Cladosporium herbarum.*

B. Thin layer chromatography

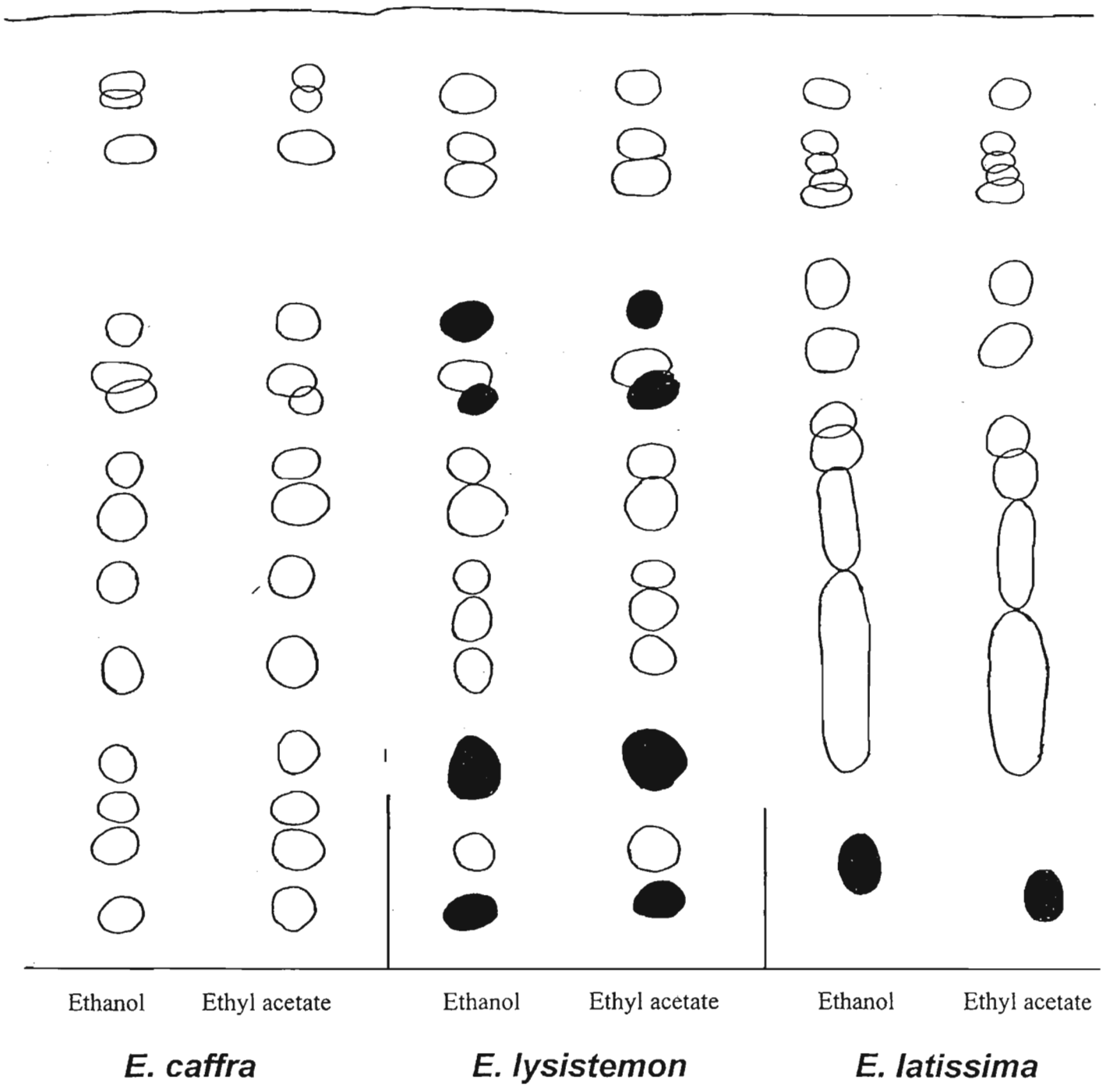


Figure 6.3.1 TLC separation of ethyl acetate and ethanol bark extracts of *E. caffra*, *E. lysistemon* and *E. latissima*.

● **Spots with anti-bacterial activity against *S. aureus***

From the TLC results (Figure 6.3.1) it is evident that there are few compounds with a substantial amount of anti-bacterial activity especially in *E. lysistemon*. There appears to be no anti-bacterial activity in the *E. caffra* extracts and only activity at one spot in the *E. latissima* extracts.

6.4 CONCLUSIONS

Bark extracts have more anti-bacterial activity than the leaf extracts specifically the bark of *E. caffra* and *E. lysistemon*. Anti-bacterial activity was mainly against the Gram positive bacteria especially *S. aureus* and *M. luteus*. The bark of *E. lysistemon* appears to be the most active against *S. aureus* (Figure 6.3.1). It therefore appears to be a good source of anti-bacterial compounds.

CHAPTER SEVEN

ISOLATION OF ANTI-BACTERIAL COMPOUNDS FROM THE BARK OF *E. lysistemon*

7.1 INTRODUCTION

Bacteria have a short doubling time if there is sufficient resources available. The basic structure of bacteria allows it to reproduce rapidly. This rapid reproduction process makes the DNA of the bacterium cell prone to mutation. The origination of a new bacterial strain is therefore an event that occurs frequently. With the advent of new bacterial strains many existing anti-bacterial compounds and substances are found to be less efficient. Even penicillin is no longer able to knock out previously susceptible micro-organisms. There is therefore a growing need to find new anti-bacterial compounds and for the design of new anti-bacterial drugs.

From Table 2.3.2 it is clear that the genus *Erythrina* is a fairly good source of anti-bacterial compounds. The results from Chapter 6 show that *E. lysistemon* has a few anti-bacterial compounds that are active against *S. aureus* (Figure 6.3.2). It is therefore a worthy undertaking to isolate anti-bacterial compounds from this plant.

7.2 MATERIALS AND METHODS

To isolate active compounds bioassay guided fractionation was carried out. Figure 7.2.1 is a summary of the methods used in the purification process.

Bulk extraction with ethanol

⇓

Vacuum liquid chromatography

⇓

Chromatotron

⇓

High performance liquid chromatography

Figure 7.2.1 Flow diagram of methods used in compound isolation

A. Bulk extraction

One kg of dried bark of *E. lysistemon* was cut into 1x1 cm pieces. These pieces of bark were placed into a Soxhlet apparatus and extracted with 2.5 l of ethanol for 30 hours.

B. Vacuum liquid chromatography

Four grams of the ethanolic extract was dried under vacuum and placed onto a Merck silica (150 g) vacuum column (30x6 cm). Compounds were eluted with 400 ml hexane : ethyl acetate. A solvent gradient was used consisting of 100% : 0% to 0% : 100% (v/v) hexane : ethyl acetate, in 5% steps. Four hundred ml fractions were collected and dried under vacuum. The residues were resuspended with acetone to a final concentration of 10 mg ml^{-1} and tested for anti-bacterial activity using the microtitre bioassay. Ten µl of each of these fractions were spotted onto a 20x10 cm plastic Merck silica gel TLC plate. The TLC plate was developed in hexane : ethyl acetate 2 : 1 (v/v).

C. Microtitre bioassay

Anti-bacterial activity was determined using the microtitre bioassay (ELOFF, 1998). The test solution (plant extract) was serially diluted 50% with water (30 µl plant extract

+ 30 µl water). An equal amount of an overnight culture of *S. aureas* that had been diluted 100 fold was added to each well. The microplate was then covered and incubated at 37 ^{0}C overnight. To indicate bacterial growth 40 µl of 0.2 mg ml^{-1} p-indonitritetrazolium violet was added to each well. After this addition the microplate was incubated at 30 ^{0}C for 30 min. Dilutions of neomycin served as a positive control. The other controls that were used was broth with no bacteria, bacterial solution with no test solution and the highest concentration of the solvent used to resuspend the plant residues.

D. Chromatotron

The sample that displayed anti-bacterial activity was dried under vacuum and the residue was placed onto a 2 mm chromatotron plate. The plate was developed in a solvent gradient consisting of 100% : 0% to 70% : 30% (v/v) benzene : ethyl acetate, in 10% steps. The total volume of the solvent mixture was 100 ml. Twenty ml fractions were collected. Ten µl of each of these fractions were spotted onto a 20x10 cm plastic Merck silica gel TLC plate. The TLC plate was developed in benzene : ethyl acetate 3 : 1 (v/v).

E. High performance liquid chromatography

The fraction that displayed anti-baterial activity was purified further on a Varian (5000) HPLC. The sample was run on a C-18 semi-preparative Phenomenex column (250x10 mm) in 80% : 20% (v/v) acetonitrile : water. The different compounds present in the samples were detected with a uv 3000HR detector at 200 nm. The flow rate was 2.5 ml min^{-1}.

F. Identification of biologically active compound

Proton and carbon NMR spectra of the active fractions were run. This was done by Professor D. Mulholland at the Department of Chemistry at the University of Natal, Durban.

7.3 RESULTS AND DISCUSSION

A. Vacuum liquid chromatography

From the TLC analysis (Figure 7.3.1) of the fractions from the vacuum column it is clear that each of the fractions contains different compounds. Fraction G appeared to be relatively clean with a single spot appearing in the hexane : ethyl acetate separation.

Figure 7.3.2 clearly indicated that the only fraction with anti-bacterial activity was fraction G. The first two wells of lane 7 showed no pink or red discoloration indicating anti-bacterial activity. The MIC of fraction G was 0.075 mg ml^{-1}. The MIC of this fraction was not as low as that of neomycin (well 15) which was 0.0013 mg ml^{-1}.

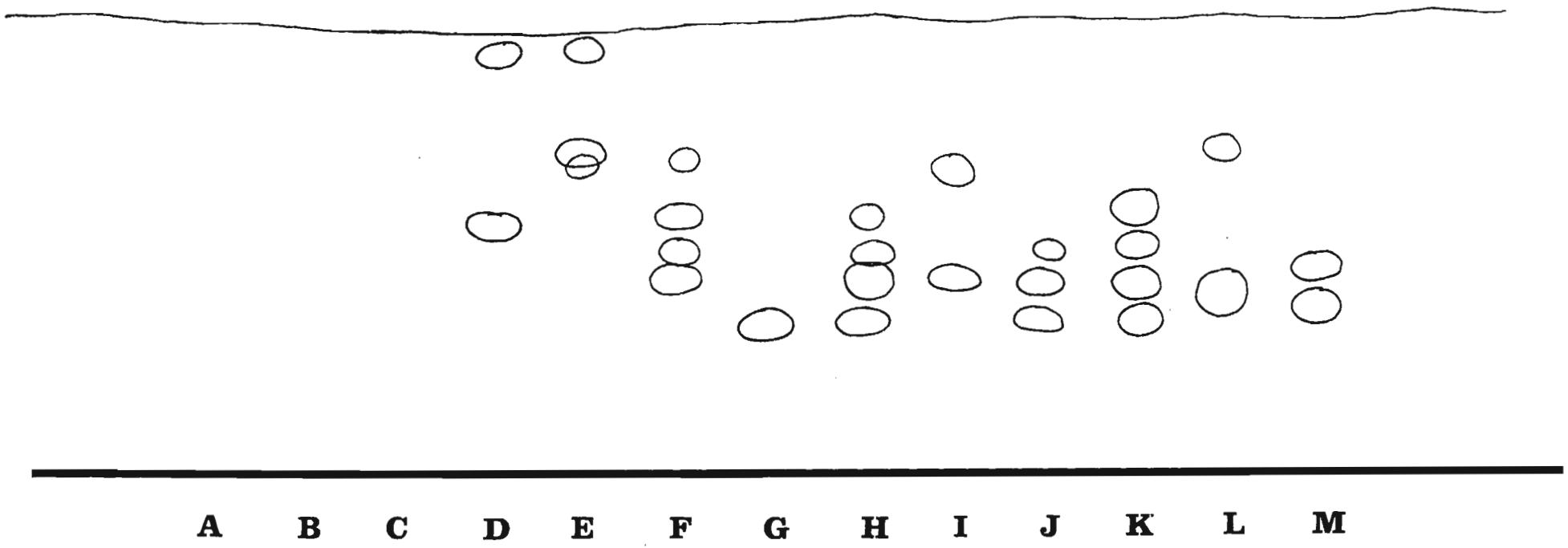


Figure 7.3.1 TLC plate of fractions from the vacuum column

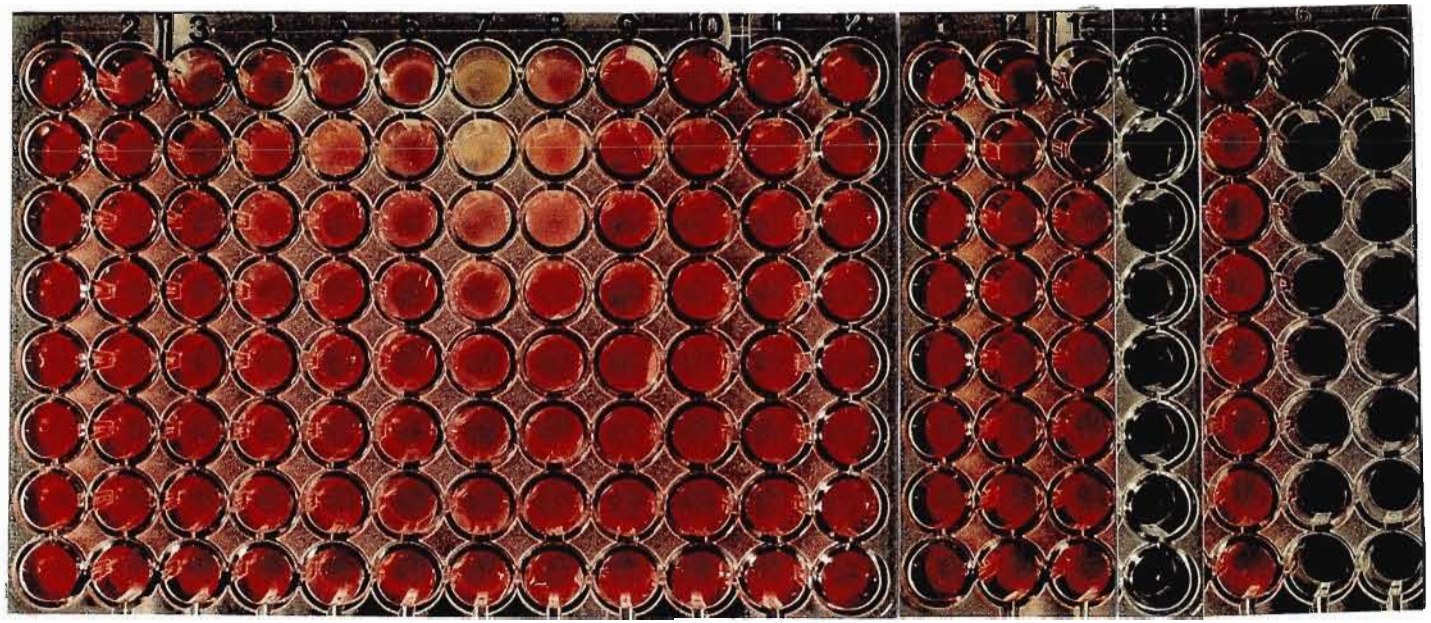


Figure 7.3.2 Microplate showing anti-bacterial activity of fractions from the vacuum column. Fractions A-M were placed into wells 1-13, respectively. Wells 14-17 were the controls (well 14 - acetone control, well 15 - neomycin control, well 16 - broth control and well 17 - bacteria control).

B. Chromatotron

Figure 7.3.5 showed that the fractions collected from the chromatotron were fairly pure with single spots appearing in fractions A, B and C in the benzene : ethyl acetate separation. The spots appeared in the same general region but the Rf values of the spots were different indicating that they are different compounds. The spot of fraction B displayed a brown colour when viewed under normal light indicating that it was definitely different from the other spots since the other spots did not show any colour.

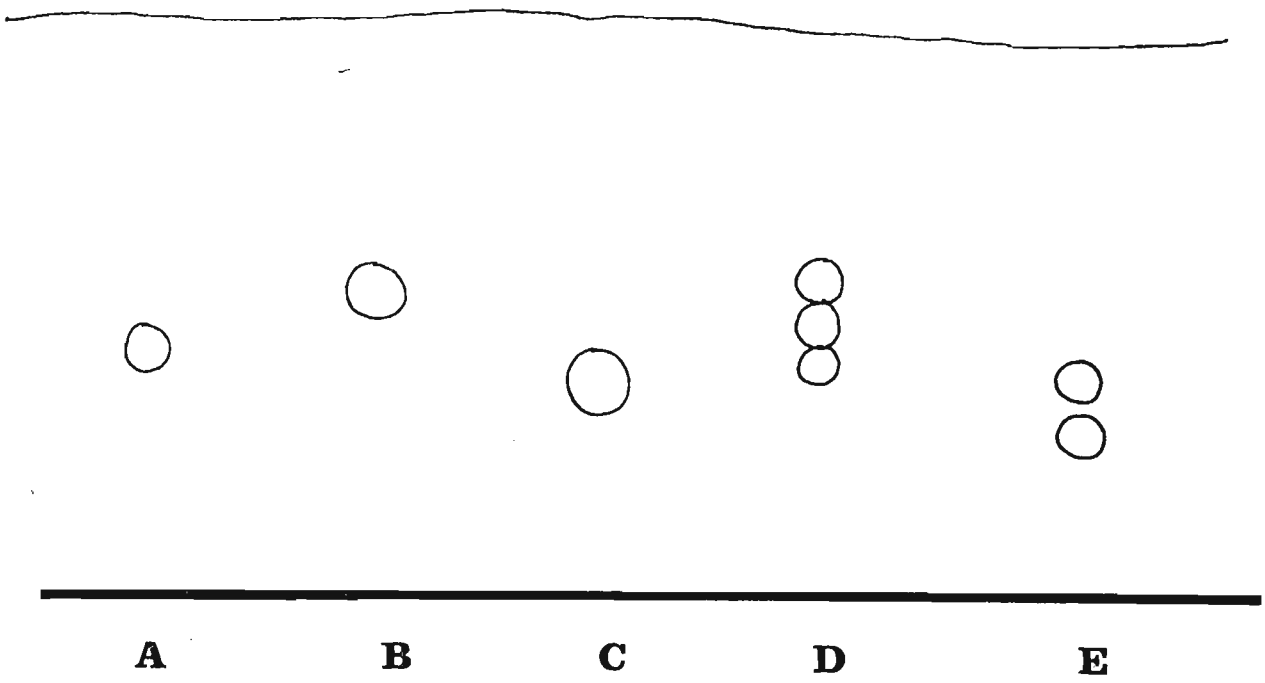


Figure 7.3.3 TLC plate of fractions from the chromatotron

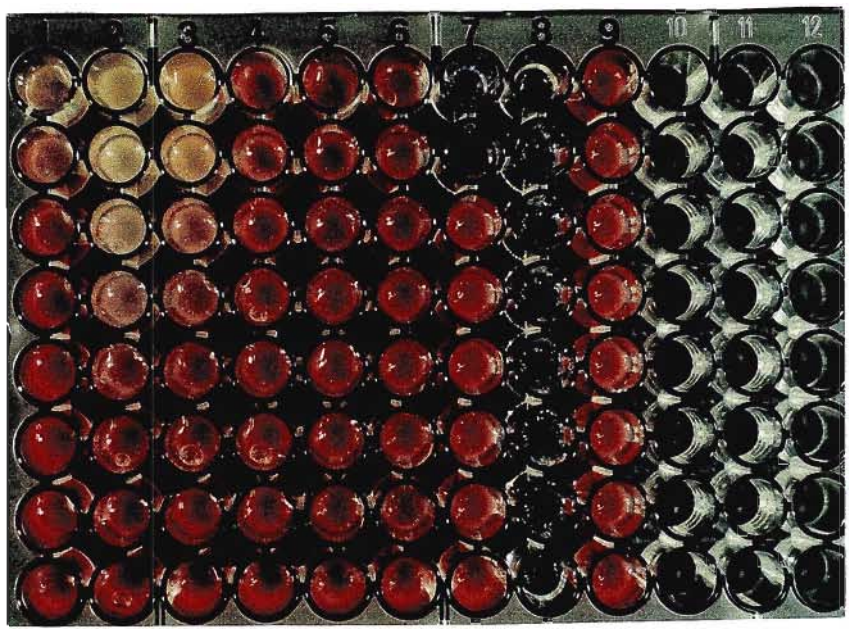


Figure 7.3.4 Microplate showing anti-bacterial activity of fractions from the chromatotron. Fractions A-E were placed into wells 1-5, respectively. Wells 6-9 were the controls (well 6 - acetone control, well 7 - neomycin control, well 8 - broth control and well 9 - bacteria control).

The microplate clearly showed that fraction B has the most anti-bacterial activity. Fraction C also showed activity but fraction B inhibited bacterial growth at a lower concentration. The MIC for fraction B was 0.0375 mg ml^{-1}. The MIC had clearly decreased from the initial one which is expected because as the sample becomes purer the active compound becomes more concentrated. Although the MIC was low it was not as low as that of the neomycin which was 0.00125 mg ml^{-1}.

C. High performance liquid chromatography

The chromatogram showed a single major peak with smaller peaks on either side of the major peak. Since the other peaks were so small it was logical to assume that they occurred in small quantities and that the concentration was to low to confer any anti-bacterial activity. Figure 7.3.6 shows that the major peak is relatively symmetrical which is an indication of its purity.

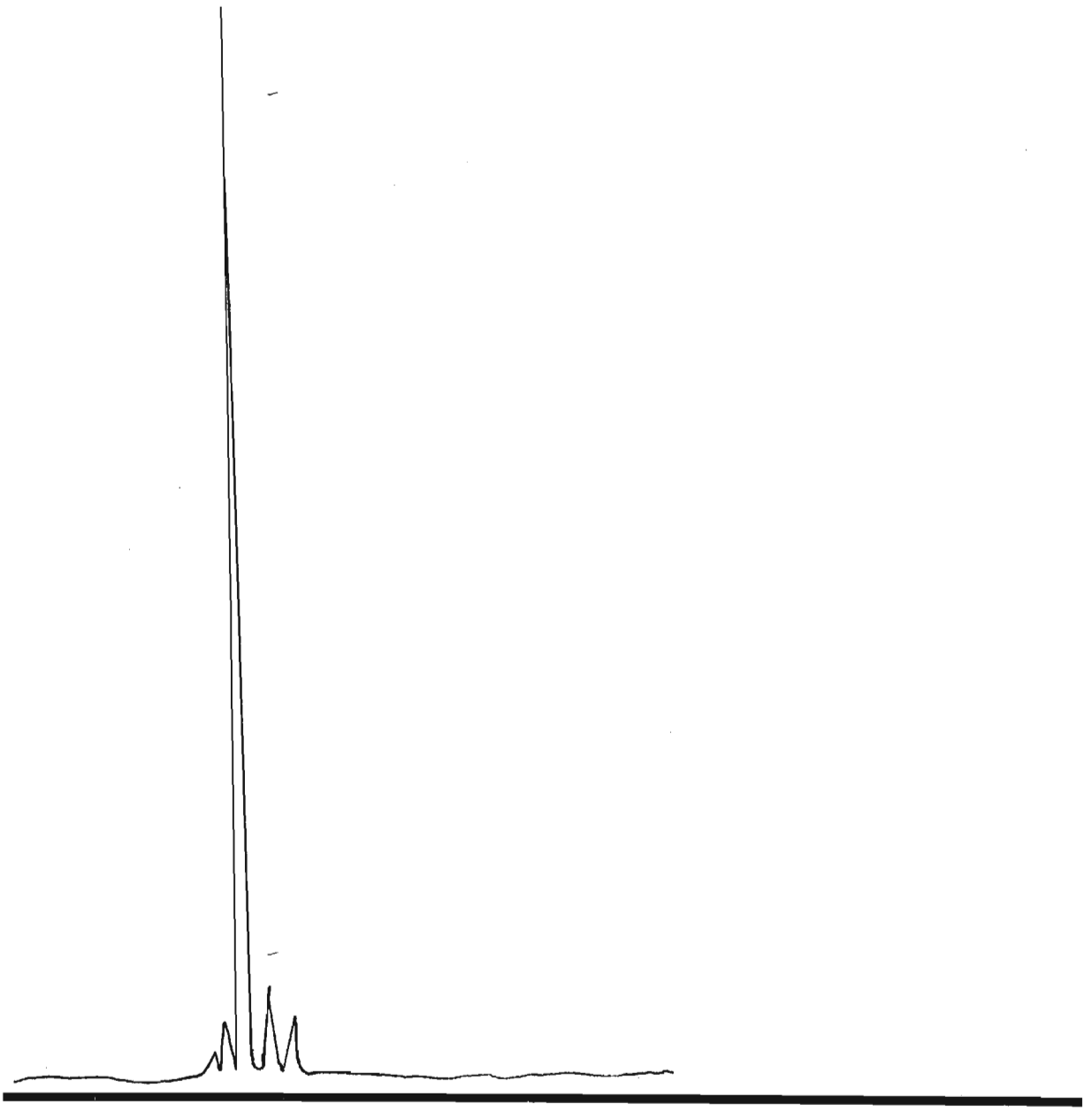


Figure 7.3.5 HPLC chromatogram of fraction B

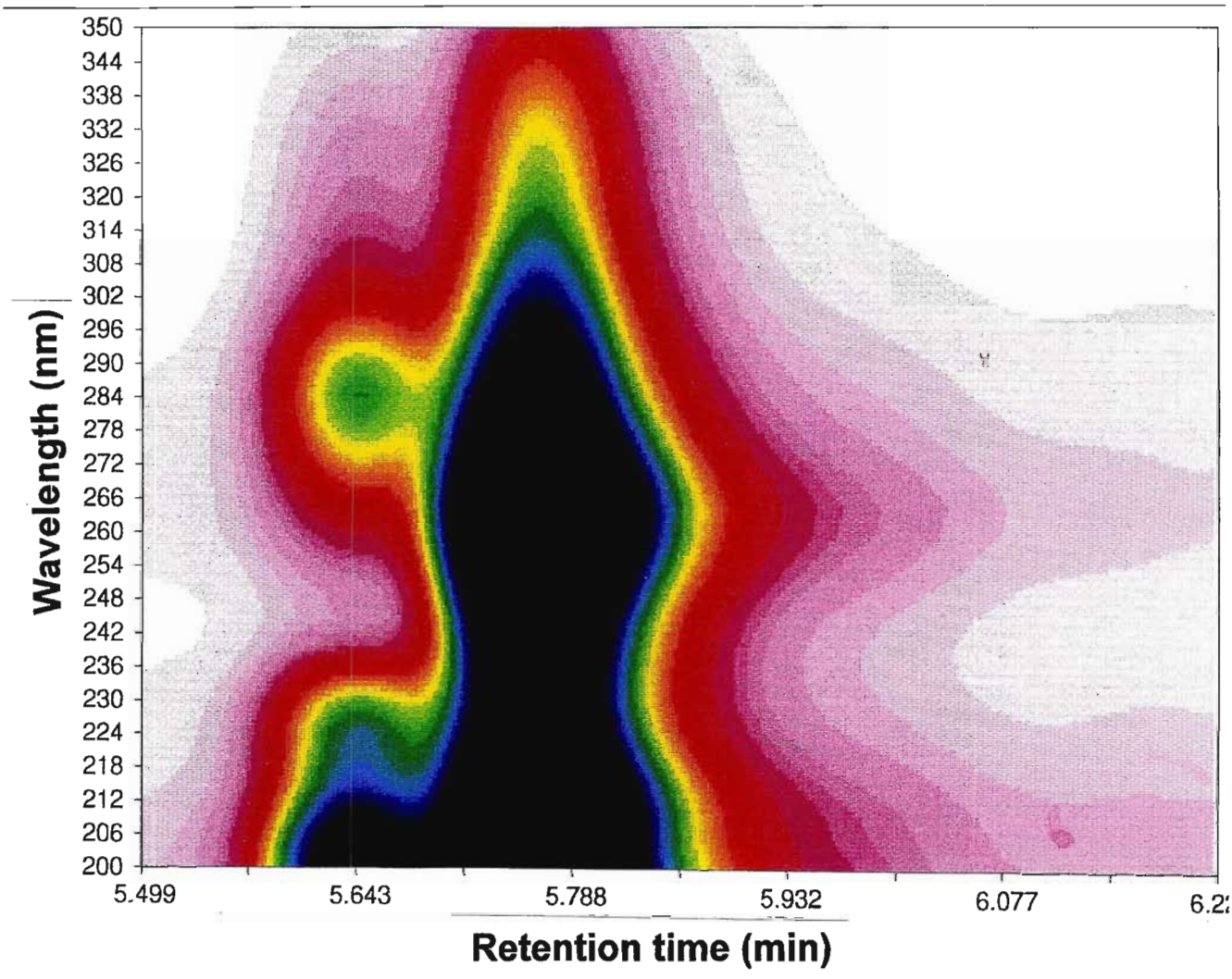


Figure 7.3.6 Ultraviolet spectrum of active compound

D. Identification of the active compound

By comparing the carbon and proton NMR to a previous study by HASHIDOKO, TAHARA & MIZUTANI (1986) it was found that the isolated compound had the following structure :

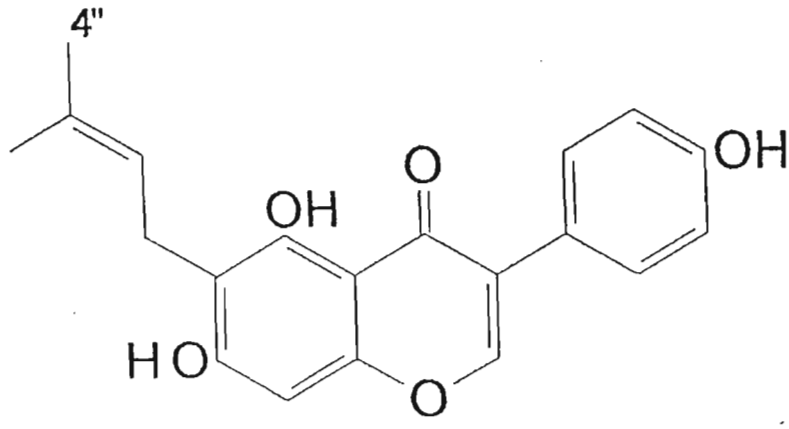


Figure 7.3.7 Wighteone

Synonyms : Erythrinin B, 6-Isopentenylgenistein and 4', 5, 7-Trihydroxy-6-prenylisoflavone

Physical description : Bright-yellow plates

Molecular formula : $C_{20}H_{18}O_5$

Molecular weight : M 338.359

Melting point : Mp 220-221 ^{0}C

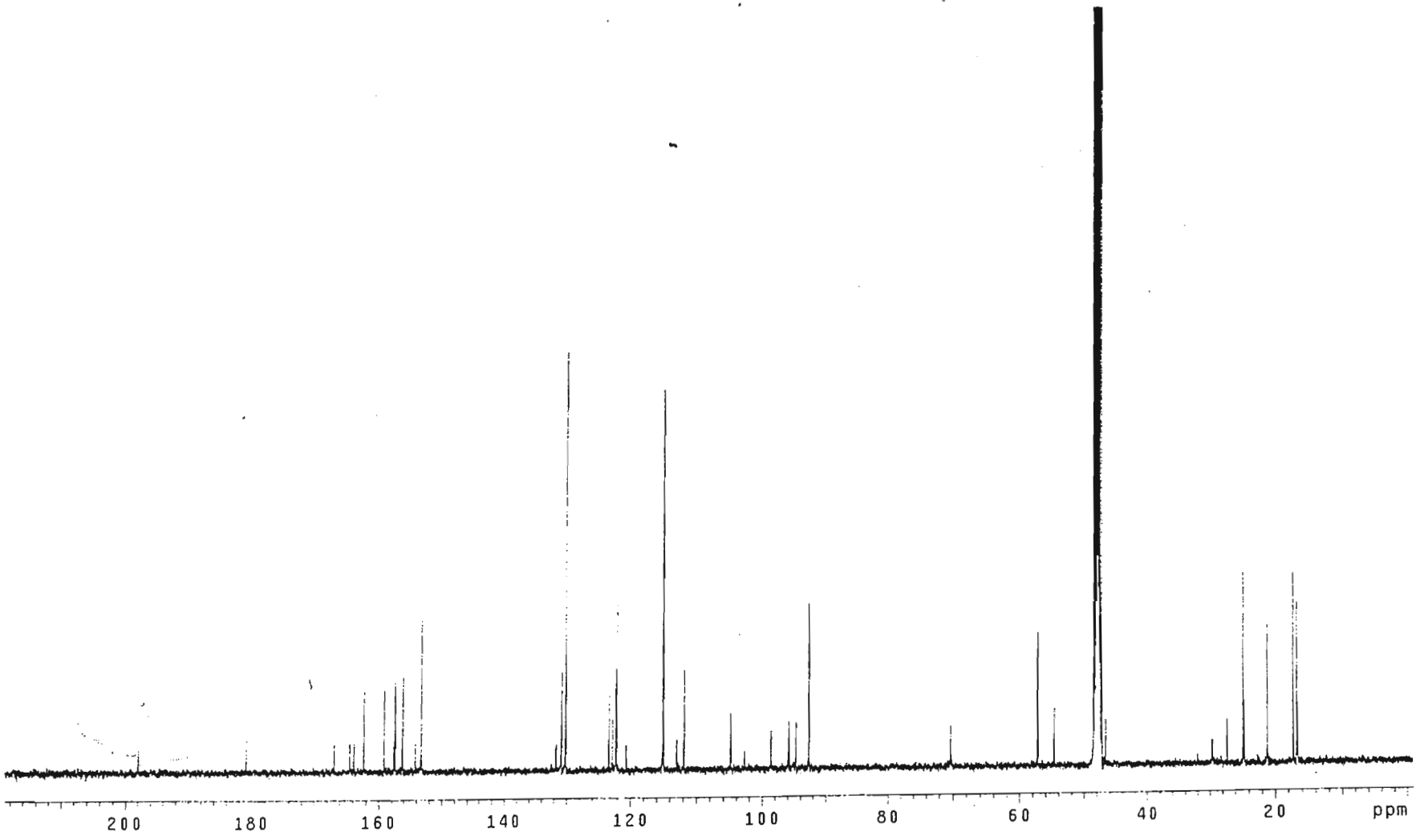


Figure 7.3.8 Carbon NMR of compound isolated from the bark of *E. lysistemon*

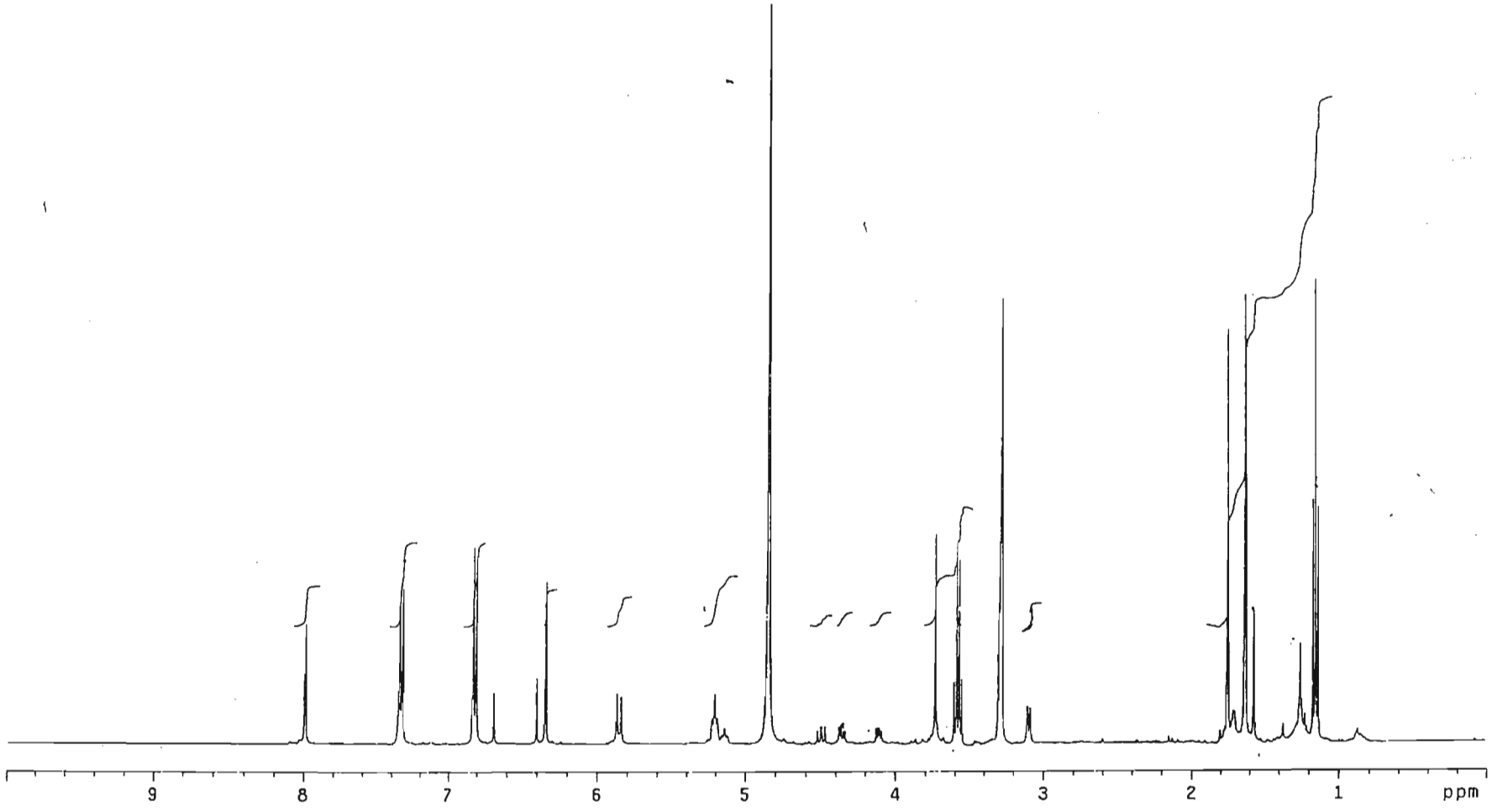


Figure 7.3.9 Proton NMR of compound isolated from the bark of *E. lysistemon*

One hundred and twenty mg of wighteone was isolated from the bark of *E. lysistemon* and the MIC of this compound was 0.038 mg ml^{-1}. The large quantity of this compound indicates that it is by no means a new compound.

This isoflavone had previously been isolated from the bark of *E. variegata* (DESHPANDE, PENDSE & PENDSE, 1977). It was previously reported that it is used as a phytoalexin. Never before has it been reported to display anti-bacterial activity. This investigation did, however, show that it does display anti-bacterial activity against the Gram positive bacterium, *S. aureus*.

S. aureus is known as the bacterium involved in food poisoning (Table 6.1.1). The food industry is one of the biggest industries worldwide, if not the biggest. There is therefore always a search to improve foods in terms of taste and preservation. The need to keep food free of bacterial growth is an important issue within the food market. It is one that receives ongoing funding and research. The anti-bacterial activity displayed by Wighteone against *S. aureus* could very well be the answer to *Staphylococcal* food poisoning. Wighteone is not a wonder drug but it could be used as a template for drug design or further investigations into compounds and drugs against *Staphylococcal* food poisoning.

7.4 CONCLUSIONS

The chromatographic methods used in this investigation proved to be successful as an anti-bacterial compound was isolated from the bark of *E. lysistemon*.

CHAPTER EIGHT
GENERAL DISCUSSION AND CONCLUSIONS

8.1 DISCUSSION AND CONCLUSIONS

The phytochemical analysis (Chapter 3) revealed that the *Erythrina* species indigenous to South Africa contains all the important secondary compounds (alkaloids, flavonoids, coumarins and triterpenes) that are found elsewhere in this genus. The TLC analysis brought to the forefront further similarities between *E. caffra* and *E. lysistemon.* These two species appear to have similar chemical profiles for the flavonoid, triterpene and coumarin detection. This is in addition to the morphological similarities that already exist between these two species.

A literature search showed that the roots, bark and leaves of these trees are used by traditional healers to cure ailments such as earache, toothache, sprains, strained ligaments, to disinfect wounds and for venereal diseases to mention but a few (Table 2.3.1). The screening investigation (Chapters 4 and 6) validated the use of the bark and leaves of *Erythrina* species indigenous to South Africa by traditional healers for inflammation and bacterial infection. The bark displayed higher anti-inflammatory and anti-bacterial activity than the leaves, specifically the bark of *E. caffra* and *E. lysistemon.* These two species are also the most widely used of the species indigenous to South Africa by traditional healers. The bark being used more frequently than the leaves.

The screening results (Chapters 4 and 6) demanded further investigation in terms of compound isolation for both anti-inflammatory and anti-bacterial activity. The anti-inflammatory compound isolation proved to be unsuccessful. This proved to be a cumbersome, frustrating process as drug discovery usually is. There has only been one report of an anti-inflammatory compound isolated from this genus (Table 2.3.2). This is in contradiction to the number of anti-bacterial compounds that have been isolated. Although the number of anti-bacterial compounds that have been isolated is small it does exceed the number of anti-inflammatory compounds by far (Table 2.3.2). The anti-

bacterial compounds that have been isolated from this genus (Table 2.3.2) appear to have activity against Gram positive bacteria, specifically *S. aureus*. The biologically active compounds appear to be flavonoids or derivatives of this family of compounds like isoflavones. The anti-bacterial compound (Wighteone) isolated from the bark of *E. lysistemon* is a prenylated isoflavone. Since there is a pronounced presence of flavonoids in the bark of *Erythrina* species indigenous to South Africa (Figure 3.3.5) one cannot help but wonder how many biologically active compounds can be extracted and isolated from *Erythrina* species indigenous to South Africa. The isolation of the anti-bacterial compound, Wighteone is by no means a major drug discovery but it does provide a new use for the compound. Drug discovery is a long and expensive process involving a lot more than is required for a Masters research project (Figure 1.3.1). Drug discovery from plants is, however not an impossibility (Table 1.3.2) and the need for drug discovery cannot be emphasized enough. In a world where disease, illness and death are the order of the day there is an overwhelming need for research of this nature to continue, on a larger scale.

8.2 FUTURE PROSPECTS

The nature of science is always to ask "how, why, when and where?". Finding or discovering something always raises more questions. A research project in reality has no end and this one has been no exception.

The use of TLC analysis should be explored to test for the presence of other types of compounds in the *Erythrina* species indigenous to South Africa. Chemical profiles of all the different plant organs should be developed and these profiles should be used as a chemo-taxonomic key to identify and distinguish between species.

The phytochemical analysis (Chapter 3) indicated the presence of alkaloids, flavonoids, triterpenes and coumarins. This analysis did, however not reveal specific chemical names of the individual compounds. Isolation of these compounds and an attempt to identify them would be a valuable project.

Literature showed that the genus *Erythrina* has a lot of biological activity. It has even been shown that some of the *Erythrina* species have anti-HIV activity (Table 2.3.2). It would therefore be interesting to screen the roots, bark and leaves for other types of biological activity, such as anti-hypertension, anti-cancer, anti-HIV, anti-malarial and anti-amoebic activity. Table 2.2.1 shows that quite a number of compounds have been isolated from this genus but Table 2.3.2 shows that very few of the compounds have biological activity. None of the compounds that have previously been isolated from the *Erythrina* species indigenous to South Africa have been reported to have biological activity. Therefore testing compounds that have been previously isolated for biological activity would be advisable.

Lastly it would be interesting to determine if the isolated anti-bacterial compound (Wighteone) is found in any of the other species indigenous in South Africa and whether it is only in the bark or also in the leaves.

We shall not cease from exploration
And at the end of all our exploring
Will be to arrive where we started
And know the place for the first time

T.S. Elliot

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