# THE MECHANISM OF THE SELECTIVE INOTROPIC AND HYPOTENSIVE EFFECTS OF TRITERPENOIDS OF PLANT ORIGIN

**By**

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# DECLARATION

I, Mwapatsa H.C. Mipando, Student Registration Number: 200101525 hereby declare that the thesis entitled:

**The Mechanism of the Selective Inotropic and Hypotensive Effects of Triterpenoids of Plant Origin,**

is the result of my own investigation and research and that it has not been submitted in part or in full for any other degree or to any other university. Where use was made of the work of others, it is duly acknowledged in the next.

31 - MAY - 2004

M.H.C. Mipando

Date

## DEDICATION

The thesis is dedicated to God for His amazing love He has bestowed on me throughout my short life on earth. I could not have done it without You in my life. Thanks for the footprints in the sand. It is also dedicated to mum and dad for seeing the vision of me being called a doctor- though you missed the details since it was a doctor of philosophy (PhD) and not medical doctor (MBBS). Lastly, I dedicate this thesis to my darling wife who had to put up with the honeymoon that never was.

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## ABSTRACT

The worldwide increasing demand for medicine from natural sources motivated our research group to search for plants with potential cardiovascular effects. Medicinal plants have been and continue to be used by over 80 % of people in the developing countries. Most of the African medicinal plants contain triterpenoids. Recent reports have shown that triterpenoids may have antihypertensive effects in rats. This result was surprising since unlike diterpenes, triterpenoids are not generally considered as hypotensive, and their cardiovascular effects have not been researched. This prompted our investigation on the cardiotonic and antiarrhythmic effects of these triterpenoids. We screened 16 African medicinal plants for their triterpenoids content and cardiovascular effects. The working hypothesis of this study was that if triterpenoids of plant origin have cardiotonic and antiarrhythmic effects in addition to their hypotensive effect, they might provide cheap and accessible treatment for hypertension complicated with cardiac failure and stenocardia in developing countries.

Upon identification and authentication of the plants by a botanist, leaves were collected and air-dried. The dried leaves were grounded before subjecting the plant material to an extraction process. We subjected the crude ethyl acetate extracts of each of the 16 plants to a further isolation and purification process that yielded four different triterpenoids namely, oleanolic acid (OA), ursolic acid (UA), uvaol (UV) and methyl maslinate (MM). The spectral properties of these triterpenoids were identical with literature values. Acute toxicity was evaluated using brine shrimp bioassay, and optimal, non-toxic dose for in vivo application was defined.

Blood pressure measurements were done using both the direct method and tail-cuff method. Urinary parameters were evaluated in urine collected from treated animals 5 and 24 hours after the administration of the test compounds. Antiarrhythmic effects were investigated in both chemically induced arrhythmia and mechanically induced arrhythmia. For chemically induced arrhythmia, we used the $CaCl_2$-, $BaCl_2$-, and adrenaline models of arrhythmia. For the ischaemia-reperfusion induced arrhythmia, we used the coronary artery ligation/reperfusion model. The cardiotonic effects of the triterpenoids and the phytochemicals were evaluated in electrically driven left atria isolated from reserpinised guinea pig and in anaesthetised rats.

All the triterpenoids and some of the phytochemicals showed a significant dose-dependent vasodepressor effect and sinus bradycardia that lasted for more than 1 hour from drug application. The test compounds had a diuretic and natriuretic effect that was comparable to that of hydrochlorothiazide. The isolated triterpenoids had a protective effect against adrenaline- and $BaCl_2$- induced arrhythmias but not on $CaCl_2$-induced arrhythmia. The protective effects were comparable to that of a class II agent for the former and class III for the latter. In terms of the ischaemia-reperfusion model, the triterpenoids conferred a protective effect that was comparable to that of Classes II and IV antiarrhythmic agents. The triterpenoids showed a positive inotropic effect that involved β-adrenergic receptors.

Therefore, evidence provided in this study indicated that triterpenoids have hypotensive, cardiotonic and antiarrhythmic activities. Since the triterpenoids have been demonstrated to be present in many of the medicinal plants of Southern Africa, the triterpenoids would provide a cheap and accessible traditional medicine source for the treatment of hypertension, complicated with stenocardia and cardiac failure.

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**Abbreviations of standard terms and units of measurement**

| | |
|---|---|
| µg | microgram |
| mg | milligram |
| g | gram |
| M | molar |
| l | litre |
| ml | millilitre |
| mm | methyl maslinate |
| OA | Oleanolic acid |
| OF | Oleuafricein |
| µl | microlitre |
| min | minute |
| s | second |
| UA | Ursolic acid |
| UV | Uvaol |
| w/v | weight/volume |
| v/v | volume/volume |
| $^{o}C$ | degrees Celcius |

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# 1.0 INTRODUCTION

In developed countries and in developing countries alike, hypertension affects 25-35% of the adult population, and up to 60-70% of those beyond the seventh decade of life (Staessen *et al.,* 2003). The World Health Organisation ranks hypertension as the third leading killer in the world since high blood pressure causes one in every eight deaths worldwide (WHO, 2002). Recent reports have shown that contrary to earlier reports, cases of hypertension are back on the increase in the United States of America (Hajjar and Kotchen, 2003). There are also reports that show that hypertension and its associated cardiovascular diseases are increasing in epidemic proportions in developing countries to the extent that it was envisaged that by 2010, cardiovascular diseases would be the number one cause of death in both developing and developed countries (Gandhi, 1997). Hypertension is rampant among blacks in Africa just as is the case amongst blacks in America (Seedat *et al.*, 1978; Seftel *et al.*, 1980; Seedat *et al.*, 1984; Greffer *et al.*, 1994; Gillum, 1996; Hajja and Kotchen, 2003). In South Africa, hypertension is one of the five major diseases that need emphasis since there is an increase in the risk factors for cardiovascular diseases. For instance, urban black Africans are consuming a lot of fat, there is an increase in smoking rates and alcohol consumption amongst them (Seftel, 1985; Yach and Townshed, 1988; Bourne *et al.,* 1993).

There have been great strides made in the diagnosis and treatment of hypertension and cardiovascular diseases. However, most of these treatments are too expensive and inaccessible to the majority of the population in the developing countries. This problem is exacerbated by the fact that most developing countries are undergoing economic problems that are resulting in reduction of budgetary spending on health services. For instance, sub-Saharan Africa has 38 of the world's 63 low-income countries where 40% of its 500

million people have less than US $1 a day to live on (World Bank report, 2002). Governments' spending on health is also undermined by debt repayment burden and cost of conflicts that are going on within the continent. Overall, most African countries have a shortage of money and face increasing restrictions and pressure in their efforts in health promotion and practices.

The economic problems facing the African population and the poor health infrastructure call for easily accessible and cheap treatment for most diseases like hypertension. This need has seen an increased interest in new therapy for hypertension from natural sources. These natural remedies would be used either as substitutes for the costly western-type drugs or as adjuvant to the western-type medicine. The obvious natural sources of treatment are the medicinal plants that are readily available in Africa. Medicinal plants with potential therapeutic values for hypertension would be both cheap and accessible to the people in developing countries. The other advantage of using medicinal plants as treatment for hypertension is that people would easily comply in taking the full dosage since the use of medicinal plants is part of their culture. It is estimated that more than 80% of the world's population relies on medicinal plants for the primary health care needs because modern drugs are either unobtainable or prohibitively expensive (Chiwuzie *et al.*, 1987; Groombridge, 1992; WHO/IUCN/WWF, 1993). Thus, despite the considerable progress made in conventional medicines and the establishment of several health institutions, traditional medicine continues to flourish and the scientific interest in it is increasing globally. It is with this in mind that the World Health Organisation (WHO) has set aside 31$^{st}$ August as the African traditional medicine day with 2000-2010 as the decade for African traditional medicine (WHO, 2003).

The increase in hypertension among Africans and the need for cheap and accessible treatment for hypertension lead our research group to look for plants with cardiovascular properties. Preliminary work was done with the leaves from African wild olive plant (*Olea europaea,* subspecies *africana*). Olive leaves have been used for treating cardiovascular diseases for centuries and its hypotensive, hypoglycaemic, antiarrhythmic, coronary vessel dilating effects have been documented (Petkov and Manolov, 1972; Ribeiro *et al.*, 1986; Zarzuelo *et al.*, 1991; Fehri *et al.*, 1994; Cherif *et al.*, 1996). These reports of the therapeutic values of the European olive leaf prompted us to check whether the African subspecies would have similar effects. *Olea europaea* subsp. *africana* is widespread throughout Southern Africa and northwards through East Tropical Africa (Neuwinger, 1994). According to Hutchings *et al.*, (1996), the plant is used in traditional medicine as a diuretic, hypotensive, emollient, tonic for urinary and bladder infections, and for headaches. The preliminary work with wild olive leaves revealed that indeed the plant had antihypertensive, antiatherosclerotic and antioxidant activities. Comparative work on *Olea europaea L.* and *Olea europaea* subspecies *africana* showed that there were chemical differences in the active principles of the two species (Somova *et al.*, 2003a). This suggested that the active principles of the African wild olive had different chemical structure and activity from that of the European olive plant.

Studies on the *Olea europaea* L. (European olive) have attributed the hypotensive, coronary vessel dilating and antiarrhythmic action to a glycoside called oleuropein. There are also reports that secoiridoid oleacin isolated from *Olea europaea* L. by fractionation, has a distinct angiotensin converting enzyme inhibitory effect (Hansen *et al.*, 1995) and an anti-oxidant activity (Bruneton, 1995). Thus, the cardiovascular effects of the extracts from the European olive leaves have been attributed to the two secoiridoids, oleuropein and

oleacein. On the other hand, the studies with the *Olea europaea* subsp. *africana* showed that the antihypertensive, antiatherosclerotic and antioxidant effects were due to the triterpenoids contained in the leaves (Somova *et al*, 2003b). The particular triterpenoids were shown to be oleanolic acid and ursolic acid. These activities of these two triterpenoids were a surprising observation since to the best of our knowledge, there is no report of the hypotensive effect that was attributed to these triterpenoids. However, Lindner *et al.,* (1978) have reported that diterpenes have cardiovascular effects.

Triterpenoids are found ubiquitously in plants (Price *et al*., 1987; Liu, 1995). Many of the African medicinal plants used in folk medicine contain triterpenoids (Hutchings, 1989). Triterpene refers to a particular type of molecular structure that has a four or five ring molecular structure containing 30 carbon atoms (Hostettmann and Marston, 1995). It is synthesized from very simple compounds (acetate units) that are found in all plants, but mainly synthesized in higher plants (flowering plants) by linking the acetate units "head to tail". The triterpenes are subdivided into about 20 groups depending on their particular structures. The base structure found in the largest variety of medicinal plants is the oleanane triterpene. This type of compound is represented by four of the most frequently occurring forms; oleanolic acid, ursolic acid, alpha-amyrin and beta-amyrin (the latter three are sometimes put in the subdivision of ursane triterpenes). There are two adverse effects of triterpenoids when the dosage is too high, and these are haemolytic effect and nauseating effect (Dharmananda, 2000).

The main biopharmacological effects attributed to triterpenoids such as oleanolic acid and ursolic acid as listed by Duke (1992) and Liu (1995) are: anti-inflammatory (Tsuruga *et al*., 1991; Simon *et al*., 1992; Han *et al*., 1997; Ringbom, *et al*., 1998; Honda *et al*., 2000);

hepatoprotective (Ma *et al.*, 1982; Saraswat *et al.*, 2000; Kuzuhara *et al.*, 2000); anti-diabetogenic (Liu *et al.*, 1994; Matsuda *et al.*, 1998; Yoshikawa and Matsuda, 2000; Taniguchi *et al.*, 2002); anti-oxidant (Balanehru and Nagarajan, 1991; Kitani *et al.*, 1999; Zhang *et al.*, 2001; Somova *et al.*, 2003); anti-hyperlipidemic (Ma, 1986; Liu *et al.*, 1987); anti-ulcer (Gupta *et al.*, 1981; Wrzeciono *et al.*,1985; Farina *et al.*, 1998); and more importantly cardiotonic and diuretic (cited by Duke, 1992). There have also been reports that oleanolic acid and ursolic acid may have anti-HIV activity (Kashiwada *et al.*, 1998 and 2000). Recently it has also been reported that oleanolic acid may have the potential of being used as a male contraceptive agent as it has been shown to induce 100 % reversible contraception in male rats and mice (Rajasekeran *et al.*, 1988; Mdhluli and Van der Horst, 2000). Triterpenoids have also been used as antispasmodic agent (Mata *et al.*, 1996).

Physiologically, a combination of the hypotensive effect of the triterpenoids as shown by our preliminary work, and the cardiotonic effect, as cited by Duke (1992), is contradictory but of high therapeutic value, ideal for treating complicated hypertension. Presently, no pharmacological drug that has both the hypotensive and cardiotonic effects exists. Thus, different drugs are used for lowering blood pressure and stimulating the heart. This makes the treatment of hypertension to be very costly. Moreover, the total number of hypertensive subjects in the developing countries is very high, and a cost analysis of possible antihypertensive drug treatment indicates that these countries can not afford the same treatment as is used in developed countries (Nissinen et al., 1988).

## AIM

The aim of this study was to investigate the cardiovascular effects of triterpenoids of plant origin obtained from South African medicinal plants.

## Working Hypothesis

We hypothesized that if triterpenoids of plant origin like oleanolic acid and ursolic acid have, in addition to their hypotensive effect, antiarrhythmic and cardiotonic effects as well, they might provide cheap and accessible traditional medicine source for treatment of complicated hypertension in developing countries.

## OBJECTIVES

1. To screen African medicinal plants with suggested triterpenoids content.
2. To find out whether there is interdependent link between the chemical structures of the triterpenoids isolated from the African medicinal plants and specific cardiovascular biological activity like hypotensive, diuretic, cardiotonic and antiarrhythmic.
3. To clarify the mechanisms of action of the above cardiovascular effects.
4. To establish whether the expected cardiovascular effects have potential therapeutic value on various experimental models of hypertension.

# 2.0 LITERATURE REVIEW

## 2.1 HYPERTENSION

Hypertension is a frequent, chronic, age-related disorder that often entails debilitating cardiovascular and renal complications (Staesssen *et al.*, 2003). Long term hypertensives often have other cardiovascular risk factors including elevated cholesterol levels, reduced high-density lipoproteins, diabetes, left ventricular hypertrophy and obesity. Hypertension imparts an increased risk of stroke, myocardial infarction, heart failure and renal failure. Many clinical trials have shown that incidences of these cardiovascular risks can be reduced by a reduction in blood pressure (August, 2003; Chobanian *et al.*, 2003). Hypertension is a complex trait that is affected by multifactor genetic and environmental factors in varying combinations rendering it difficult to treat. Cohn (1998) defines hypertension as a state of abnormal arterial function and structure associated with endothelial dysfunction, vascular smooth muscle constriction or remodelling, increased impedance to left ventricular ejection and propensity for atherosclerosis, often but not always manifested by an elevated blood pressure. Human hypertension is usually a slowly developing disorder of middle to old age that predisposes to cardiovascular disorders that cause most of the morbidity and mortality in the elderly. The incidence and sequela of hypertension vary markedly depending on the subgroup of the patient for instance gender and race (Cooper and Kaufman, 1998; O'Donnell and Kannel, 1998). In the USA, it has also been shown that during the past decade there was a high proportion of hypertensive people in non-Hispanic blacks than in any other race, and that women had less incidences of hypertension than males (Hajjar and Kotchen, 2003).

Human hypertension is probably triggered by environmental influences acting on a genetic predisposition. Examples of these environmental influences are; physical inactivity, excess sodium intake, excess alcohol consumption, obesity particularly visceral, acquired or congenital reduction of nephrons number (Barker *et al.*, 1992; Hall *et al.*, 1992; Barenbrock *et al.*, 1995; Kaplan, 1995; Woelk, 1995; Rosmond and Björntorp, 1998; Keller *et al.*, 2003). Specific genes responsible for hypertension have not been identified though there has been a lot of break through in the past decade that have led to the discovery of 17 human genes that cause mendelian forms of either hypertension or hypotension (Nabel, 2003; Staessen *et al.*, 2003).

In general there are two kinds of hypertension, namely essential also known as primary hypertension and secondary hypertension. Essential hypertension is loosely defined as an elevated blood pressure above a predetermined cut off point that result from an unknown underlying cause. On the other hand, secondary hypertension results from an underlying, identifiable and often correctable cause (Onusko, 2003). According to the World Health Organisation as well as the American National Heart, Lung and Blood institute, a person is considered to be hypertensive if their average systolic blood pressure is more than 140 mm Hg and/or their average diastolic blood pressure is more 90 mm Hg (WHO Guidelines, 1999; Chobanian *et al.*, 2003; Haggar *et al.*, 2003). For patients with diabetes or chronic kidney disease, the cut off point for systolic blood pressure is 130 mm Hg whilst the for diastolic blood pressure is 80 mm Hg (Chobanian *et al.*, 2003; Haggar *et al.*, 2003)

### 2.1.1 Secondary Hypertension

Only about 5 to 10 % of hypertension cases are thought to result from secondary causes. Secondary hypertension is curable because once its cause is identified it can be treated.

Some of the causes of it are; Cushing's syndrome, coarctation of the aorta, diseases of the kidneys and urinary tract like nephritis, diabetes, amyloid contracted kidney, chronic pyelonephritis, disease of the renal arteries, obstructive sleep apnoea, aldosteronism, endocrine disorders, excess catecholamines, excess erythropeietin and drugs (Pickering, 1995; Onusko, 2003). In most cases, the above causes can lead to pulmonary hypertension or renal hypertension.

### 2.1.2 Essential Hypertension

Essential hypertension affects around 90 % of the hypertensive population. Although it is said to have no known cause, its pathophysiology depends on a number of factors like the inability of the kidney to excrete electrolytes like sodium at a normal blood pressure, malfunction of the central nervous system, endocrine factors and diseased blood vessels (le Noble *et al.*, 1998; Staessen *et al.*, 2003). Blood pressure deregulation is also caused by genetic factors, both monogenic and polygenic factors. These genetic factors are said to contribute approximately 30 –50 % of the variation in blood pressure between individuals (Dominiczak *et al.*, 1998). It is also thought that in essential hypertension, the number of nephrons is reduced though it is not known what determines this reduction though the intra-uterine events are thought to play a major role (Ingelfinger, 2003; Keller *et al.*, 2003). From here on hypertension will refer to essential hypertension.

## 2.2 DIAGNOSIS OF HYPERTENSION

Hypertension is diagnosed mainly through the measurement of blood pressure in combination with other parameters like electrocardiogram, urinalysis, complete blood

count and blood chemistry profile (e.g. potassium, sodium, creatine, fasting glucose, fasting lipid levels). These parameters determine the cardiovascular risk factors that are associated with hypertension. Although new methods of assessing hypertension are being introduced, conventional blood pressure measurement remains the standard method of determining hypertension.

As said before, a blood pressure reading of more than 140/90 mm Hg is defined as hypertensive. This reading is supposed to be an average of at least two reading taken on different times. Hypertension is further classified into stages (refer Table. 1).

## 2.3 REGULATION OF BLOOD PRESSURE

Arterial blood pressure is the product of cardiac output times total peripheral resistance. It is monitored continuously by baroreceptors in the cardiopulmonary region and in the carotid sinus. The baroreceptors transmit their information to the vasomotor center in the central nervous system. The vasomotor center in turn changes afferent sympathetic tone, regulating cardiac output and peripheral vascular resistance (Osswald and Mühlbauer, 1995).

The physiological regulatory systems for blood pressure include neurotransmitters and humoral factors acting on the cardiac and vascular smooth muscle and endothelium. Excessive vasoconstriction, commonly involving the endogenous peptides, angiotensin II, endothelin, or deficient vasodilatation, often involving nitric oxide (NO), are common mechanisms in hypertension. Functional changes that have been implicated to play a role in hypertension include structural vascular changes, sodium retention by the kidneys,

increase in sympathetic activity, abnormalities in the rennin-angiotensin system and the integrity of the endothelium.

Table 1 Classification of blood pressure

| Category | Systolic blood pressure (mm Hg) | Diastolic blood pressure (mm Hg) |
|---|---|---|
| **Normotension** | | |
| Optimal | < 120 | < 80 |
| Normal | < 130 | < 85 |
| High normal (prehypertesive) | 130 – 139 | 85 – 89 |
| **Hypertension** | | |
| Stage 1 (mild) | 140 – 159 | 90 – 99 |
| Stage 2 (moderate) | 160 – 179 | 100 – 109 |
| Stage 3 (severe) | 180 – 209 | 110 – 119 |
| Stage 4 (malignant) | ≥ 210 | ≥ 120 |
| Isolated systolic hypertension | ≥ 140 | < 90 |

Adapted from Staessen *et al.*, (2003) with slight modifications.

## 2.4 MODELS OF HYPERTENSION

Since most cases of hypertension are of unknown causes, it is difficult to study the underlying causes in humans. Thus, in additional to clinical studies of hypertension, most of the studies are done in animals in what is called experimental hypertension using animal

models. As hypertension is a complex trait due to a combination of genetic and environmental factors, it is impossible to study all the possible causes in just one model and as such different models have and are being developed to study a particular aspect of hypertension. Animal models have allowed the study of hypertension and cardiovascular diseases in the early stages as well as the investigation of the mechanisms of the pathogenesis and the effects of drug intervention. These models have their advantage over humans because they include complete genetic homogeneity, large number of progeny can be obtained, and it is possible to produce large genetic crosses. In addition, an investigator can tightly control the environmental influences unlike in humans. The animals that are mostly used are dogs, rabbits, cats and rats. The rat is mostly preferred over the other animals because its use is rational from the economical viewpoint and for the fact that many techniques have been developed to measure relevant functional parameters. Rat models of hypertension developed by selective breeding over many generations have been in use for a long time (Smirk and Hall, 1958; Dahl, Heine and Tassinari, 1962; Rapp, 1982). On the other hand, rat models of hypertension posses a number of problems. For instance, cardiovascular diseases like hypertension and heart failure in humans usually slowly develop with wide ranging neurohumoral adaptations in contrast to the acute onset of symptoms in many surgical or drug induced rat models of these diseases. Secondly, hypertension is uncommon in young humans but markedly increases with age yet most models of hypertension use young adult rats (Doggrell and Brown, 1998).

### 2.4.1 Spontaneously hypertensive rats (SHR)

This is the most used model of cardiovascular disease with the wistar Kyoto rat (WKY) as the normotersive control. SHR are descendants of an outbred wistar male with

spontaneous hypertension from a colony in Kyoto, Japan, mating with a female with elevated blood pressure (Doggrell and Brown, 1998). The brother vs. sister mating is continued with selection for spontaneous hypertension, defined as a systolic blood pressure of over 150 mm Hg persisting for more than one month. As in humans, hypertension becomes more severe in males than females. Thus, male SHRs are commonly used as a model of established human hypertension.

SHR has the disadvantage of only modelling one of the many possible causes of human hypertension due to its lack of inter-individual variation. However, it has a number of advantages for studying human hypertension. Firstly, it is useful for mapping and identifying genes responsible for developing hypertension since it is an inbred genetic model. Secondly, it follows the same progression of hypertension as human hypertension with pre-hypertensive, developing and sustained hypertensive phases with each phase lasting at least several weeks. Lastly, the SHR is a useful model since compounds that lower blood pressure in SHR also lower blood pressure in hypertensive humans and as such, it is used to test new antihypertensive medication (Doggrell and Brown, 1998).

### 2.4.2 Stroke-prone Spontaneously hypertensive rats (SHR-SP)

The SHR-SP was developed to study stroke since hypertension is the major risk factor for stroke. These rats are hypertensive at five weeks and systolic blood pressure rises to at least 250 mm Hg in males. Salt loading has been shown to accelerate the development of hypertension and the occurrence of stroke (Okamoto, Yamori and Nagaoka, 1974). The disadvantage of this model according to Doggrell and Brown (1998) is that the rats die at a

very young age and as such, the model misses out the common age at which strokes are common in humans, which is above the fourth decade of life.

### 2.4.3 Dahl/Rapp salt-sensitive rats

Understanding salt-sensitive hypertension has been helped by the introduction of the inbred rat models, especially the Dahl/Rapp salt-sensitive and salt-resistant rats. The development of hypertension and heart failure in these rats can be controlled by titration of the amount of salt in their diet. As is the case in most rat models of hypertension, the development of hypertension is more rapid and greater in males than female rats. When both the salt-sensitive and the salt-resistant strains are fed a higher salt diet (8 % NaCl), the salt-sensitive strain express a significantly increased sodium and water retention, and an increased activity of the sympathetic nervous system (Dahl *et al.*, 1962; Rapp, 1982; Somova, Channa and Khan, 1999). It has also been shown that the adrenal gland of the Dahl salt-resistant strain produce less 18-hydroxydeoxycorticosterone (18-OH-DOC) than that of the salt – sensitive strain and that the circulating levels of 18-OH-DOC were lower in the former (Kurtz, 1995). These genetically induced alterations in the levels of 18-OH-DOC are thought to contribute to the relative salt resistance of the Dahl salt-resistant strain.

This model therefore provides an interesting model for the interaction of an environmental factor (salt in this case) with genotype. This is a more relevant model to study hypertension in sub-Saharan Africans where studies have shown that most of the hypertension amongst the population is salt-sensitive (Mufunda and Somova, 1993). It has also been shown that blacks retain a lot of sodium and that there is a greater prevalence of salt sensitivity among blacks compared with white adults and children (Luft *et al.*, 1991;

Wilson *et al.*, 1992; Edwards, 1995). It has also been shown that in black adolescents, boys are most likely to be identified as salt sensitive based on sodium load than girls (Wilson *et al.*, 1996). The other advantage with this model is that it is easy, non-invasive, relatively quick and consistent thereby making it easy to be used by any laboratory without any need of expertise.

### 2.4.4 Diabetic hypertensive rats

Diabetes is an important risk factor in patients with hypertension and heart disease. The common model for diabetes in rats is obtained by administering streptozotocin to adult rats. Rapid injection of streptozotocin to adult rats produces many of the characteristic cardiovascular and renal features of humans with uncontrolled insulin-dependent diabetes (type 1 diabetes mellitus). Other studies have injected streptozotocin to spontaneously hypertensive rats (SHR) to produce a model of hypertensive insulin-dependent diabetic humans (Doggrell and Brown, 1998). Streptozotocin selectively destroys Beta (β) cells of the pancreas (Grussner *et al.*, 1993) thereby impairing insulin production leading to hyperglycaemia. Any changes occurring thereafter are considered related to the induced hyperglycaemia. Complications of diabetes mellitus such as impairment of kidney function can therefore be studied using this model. However, this model is criticized for not being appropriate for most humans since most diabetic patients suffer from non-insulin dependent diabetes (type 2 diabetes mellitus). Thus, the more relevant model to type 2 diabetics is the genetically determined obese Zucker rat or the Otsuka Long-Evans Tokushima fatty rats (Doggrell and Brown, 1998). These two models develop mild hypertension with typical cardiac and renal complications.

### 2.4.5 Transgenic Rats

Transgenic techniques are now increasingly being used since they offer the possibility of analysing responses to selected genes. In transgenic animals, overexpression or suppression of individual candidate genes involved in hypertension permits a systematic testing of gene function, regulation, and its relationship to a particular phenotype (Hoffmann *et al.*, 1995). Transgenic rats develop hypertension, hypertrophy and heart failure. The commonly used species for transgenic experiments is the mice. An example of a transgenic model is the murine Ren-2 gene that has been used to generate transgenic rats to study the rennin-angiotensin system and its contribution to the cardiovascular system. Male rats have a sustained angiotensin II- dependent increase in blood pressure with low circulating rennin levels (Hoffmann *et al.*, 1995; Doggrell and Brown, 1998).

According to Staessen *et al.*, (2003), some experts favour the whole genome approach and exploit synteny between species in the search for major genes that could lead to hypertension. Inbred rats and genetically engineered mice, in which gene activities have been specifically manipulated, produce new insights in the long-term regulation of blood pressure. However, these rodent models do not stand comparison with the complexity and heterogeneous nature of the human disease.

### 2.4.6 Models for studying renovascular hypertension

The kidney has long been known to be vital in cardiovascular homeostasis though renal damage is a relatively minor cause of human hypertension. The importance of the kidney in hypertension has recently been emphasized in studies of the number of nephrons in

relation to hypertension. In this study, it was observed that white hypertensives have very few nephrons than normotensive subjects (Keller *et al.*, 2003). Renovascular hypertension develops in response to renal ischaemia and models for it involve restricting blood flow by clips on the renal arteries as first described in the classical Goldblatt experiments (Goldblatt, 1995).

The three common models are; Firstly, the one-kidney one clip model (1K1C) that involves the removal of one kidney and the placement of a clip on the renal artery of the remaining kidney. Secondly, there is the two-kidney two clip model (2K2C) that involves clipping the renal arteries of both kidneys. This model mimics the bilateral renal artery stenosis in humans. Thirdly, there is the two-kidney one clip model whereby one clip is placed on the renal artery of one of the kidneys. The contra lateral kidney remains untouched. The major problem with the above models is that they induce a sudden increase in blood pressure unlike the slow onset of human hypertension.

### 2.4.7 Models for Pulmonary hypertension

In human pulmonary hypertension, pulmonary artery systolic and mean pressures exceed 30 mm Hg and 20 mm Hg respectively at rest whilst during exercise the pulmonary artery mean pressure exceeds 30 mm Hg. Some of the causes of pulmonary hypertension are chronic hypoxia, congenital heart defects, autoimmune disease, thromboembolism, left sided heart failure, ingestion of anorexigens and over expression of angiopoietin-1 in the adult lung (Du *et al.*, 2003).

There are two commonly used rat models for pulmonary hypertension and these are the monocrotaline model and the hypoxia model. In the former model, a subcutaneous administered single dose of a crotalaria alkaloid, monocrotaline, is made to rats. It is used as a non-invasive, slowly developing, and haemodynamically relevant model for pulmonary hypertension. In the hypoxia model, hypoventilation in rats by prolonged exposure to normobaric hypoxia is used to induce pulmonary hypertension. Hypoxia in rats produces pulmonary vascular remodelling and an increase in endothelin, a vasoconstrictor, resulting in hypertension. This model mimics the human disease better since alveolar hypoxia is often a stimulant of the pulmonary vasoconstriction that underlies the hypertension seen in humans ((Doggrell and Brown, 1998).

The above rat models of hypertension are not exhaustive as there are other models that have not been mentioned here. Examples of those not mentioned are; Deoxycorticosterone acetate (DOCA) rats, Milan hypertensive strain (MHS), Lyon hypertensive rats (LH) and New Zealand genetically hypertensive rats (GH).

## 2.5 MEASUREMENT OF BLOOD PRESSURE IN EXPERIMENTAL HYPERTENSION

The recording of blood pressure in rats is one of the most widely used techniques in experimental hypertension. Experimental hypertension, defined as a sustained and long-lasting blood pressure elevation, can only be shown to exist by measuring blood pressure repeatedly in the same animals. Preferably, the measurement should be done while the animals are conscious to avoid artefacts due to anaesthesia (Buñag, 1991). Rats are the

preferred animals of choice due to amongst other reasons, their uniformity in body size and genetic background, relatively low costs, and the availability of several models of experimental hypertension as has already been described above. In principle, there are two techniques for measuring blood pressure in rats, that is the indirect measurement using the tail-cuff method, and the direct recording via arterial catheters.

### 2.5.1 Indirect blood pressure recording

All indirect methods require the use of a cuff for occluding the blood flow in a peripheral artery and thus this method is related to that used in humans. The blood pressure can be taken either from the rat tail or from a hind leg. The tail is the preferred area since rats use their tails for regulating body heat. This makes the tail vessels to aptly respond to stimuli that increase sympathetic vasomotor tone to induce localised vasoconstriction (Buñag, 1991).

In principle, an annular occluding cuff applied to the base of the rat tail is inflated to occlude the arterial blood supply. Upon slowly deflating the cuff, the reappearance of pulsation (signifying resumption of blood flow) is taken as systolic blood pressure. Systolic blood pressure can also be determined when the pulsation disappears at inflating cuff pressure. Different sensors have been employed to identify pulsatile blood flow in the tail artery. These sensors operate based on plethsmographic instruments, microphones, ultrasonic sensors, piezo crystals, photoelectric devices, infrared-light sensors, or impedance techniques. In general, the sensor has to be sufficiently sensitive to detect the first arterial pulsation that appears as the occluding cuff is gradually deflated (Buñag, 1991; Sponer *et al.*, 1993).

With most tail-cuff methods, unanaesthetised rats have to be restrained and preheated during measurements. This may affect the reliability of the readings since restraint and heat may elicit substantial pressor effects. Thus, in order to get reliable pressure values, the rats need to be accustomed to the conditions of pressure recording by means of a training program. The training consists of all parts of the procedure such as handling, restraining, warming up, and cuff-pressure increase and decrease. Most laboratories train their rats for five consecutive days prior to the starting of the actual experimental pressure readings. In this way, the rats become familiar with whole procedure thereby minimising stress reactions, and thus obtaining reliable pressure values.

The advantages of using the tail-cuff method are firstly, that no surgical procedure is required before starting blood pressure reading. This makes it possible for the method to be used by anyone. Secondly, the method is appropriate for chronic experiments since it does not confer any serious risk to the health of the animal. On the other hand, the major limitation with this method is that it does not provide continuous recording of blood pressure. This makes it difficult for the method to be used in some pharmacological investigations where one might miss the peak effect of a drug in particular those with a short duration of action.

When monitoring drug-induced responses it is advisable not to solely rely on the tail-cuff measurements due the possible errors that occur with this method. Therefore to verify the blood pressure values, it is advisable to validate the readings by comparing the values obtained simultaneously by the tail cuff method with those obtained by the direct method in

the same rats (Buñag, 1991; Sponer *et al.*, 1993). Each laboratory is supposed to do its own validation whenever possible.

### 2.5.2. Direct blood pressure recording

Intra-arterial pressures can be recorded continuously by inserting catheters made of small-bore tubing into suitable arteries like the carotid, iliac, femoral, caudal or renal arteries (Sponer *et al.*, 1993). The catheter is then connected to a mercury manometer or pressure transducer.

The major advantage of this method is that the whole procedure can be done under almost physiological conditions with no requirement for warming, restraining or stress. The method also allows continuous recording of blood pressure. This make it appropriate for registering any rapid changes of blood pressure caused by the test drugs thereby allowing the determination of their peak effect. On the other hand, the method is not appropriate for chronic studies because of the risk to the healthy of the animal introduced by the surgical procedure. It is also unattractive because it requires trained technicians to prepare and implant the catheters.

In order to reproduce authentic blood pressure values, a number of principles need to be followed. Firstly, the size and shape of the catheters must be adapted to the body weight of the animal as well as the artery where they are going to be implanted. Normally, rigid-walled tubings (polyethylene or Teflon) are used to make catheters. Secondly, clot formation can be reduced by filling the catheters with dilute heparin solution and also all tubes, plugs and needles must fit exactly. Thirdly, during the blood pressure measurement,

the whole tubing system is supposed to be free of air bubbles to avoid dampening of pulse pressure (Buñag, 1991; Sponer *et al.*, 1993).

## 2.6- CURRENT THERAPY FOR HYPERTENSION

Before starting treatment, there is a need to assess blood pressure level and the absolute risk of cardiovascular disease. Depending on the assessment, intervention can either be through lifestyle modifications or/in conjunction with drug treatment. Treatment can be with lifestyle modifications alone for up to one year if there are no other risk factors. Drug treatment is then administered if there is no improvement with the lifestyle modifications. On the other hand, patients with stage 2 hypertension or more, or those with high cardiovascular risks, and patients with diabetes, drug therapy is indicated.

### 2.6.1 Intervention through lifestyle modifications

Evidence suggest that sodium restriction; maintaining adequate intake of dietary potassium, calcium and magnesium; stopping excessive alcohol consumption; restriction in caloric intake while eating abundant fruits, vegetables and low-fat dairy products; weight loss in obese people; regular dynamic exercise, and abstaining from smoking might reduce blood pressure and cardiovascular risks (Opie and Steyn, 1995; August, 2003;). Life style modifications are encouraged since they are safe and inexpensive though the major problem with them is their low long-term sustainability.

### 2.6.2 Intervention through drug treatment

Drug treatment is used on the basis that the medication will decrease blood pressure and as a result prevent the cardiovascular and renal complications associated with raised blood pressure. The established drugs are classified into six broad classes depending on their mechanism of action. The classes are; diuretics, beta-blockers, angiotensin-converting enzyme (ACE) inhibitors, calcium- channel antagonists, alpha-blockers and angiotensin – receptor antagonists. These drugs are administered either as monotherapy or as a combination therapy depending on the stage of hypertension. There is still a lot of controversy as to which drug is better than the other.

Based on evidence from clinical trials even in Africa, it is suggested that low dose thiazides (a diuretic) might be the most cost-effective way to start pharmacological treatment in most patients (Man in't Veld and van den Meiracker, 1995; Opie and Steyn, 1995; Seedat, 1999; Seedat, 2000). It worthy noting that for each class of antihypertensive drug, indications or contraindications exists. For instance, stable heart failure is an indication for thiazides, aldosterone receptor blockers, β-blockers, or angiotensin converting enzyme (ACE) inhibitors but not for angiotensin receptor blockers. A history of myocardial infarction favours the use of β-blockers or ACE inhibitors while renal impairment, microalbuminuria, or proteinuria is an indication for ACE inhibitors or angiotensin receptor blockers (Wing *et al.*, 2003). Systolic hypertension warrants the use of thiazides or long – acting dihydropyridines (Staessen *et al.*, 2003). Black people with hypertension respond better to thiazides or calcium – channel blockers than to inhibitors of the renin system, in terms of both blood pressure reduction and prevention of complications associated with it. It has been shown that black South Africans, just like other blacks, do not respond well to β-

blockers or angiotensin converting enzyme (ACE) inhibitors but rather to diuretics and/or $Ca^{2+}$ antagonists (Seedat 1989; Middlemost *et al.*, 1992; Materson *et al.*, 1993; Middlemost *et al.*, 1994; Skoularigis *et al.*, 1994; Opie and Steyn, 1995; Seedat, 1999; Seedat, 2000)

## 2.7- ARRYTHMIA

Arrhythmias are disturbances in the heart rate and rhythm that may be due to abnormalities in the impulse formation or impulse conduction. Impulses may be initiated abnormally (1) by slow diastolic depolarisation of automatic cells in ectopic sites or (2) by afterdepolarisation that reach threshold (Berne and Levy, 2001). On one hand, ectopic foci comes into play when the automatic cells in atrium, atrioventricular (AV) node, or His-Purkinje system initiate propagated cardiac impulses either because the sinoatrial node is suppressed, or because the rhythmicity of the ectopic foci is abnormally enhanced. On the other hand, under abnormal conditions, afterdepolarisation may appear either early or late into the phase 3 of a normally initiated beat, or the beginning of phase 4. If these afterdepolarisation reach threshold they may themselves trigger propagated impulses.

Disturbances of impulse conduction consist of conduction block and re-entry. In conduction block there is failure of propagation in a cardiac fibre as a result of a disease process due to a number of reason for instance, ischaemia, inflammation, scar tissue or calcified portions of the heart, or a drug. On the other hand, in re-entry a cardiac impulse may traverse a loop of cardiac fibres and re-enter previously excited tissue when the impulse is conducted slowly around a loop and the impulse is blocked unidirectionally in some section of the loop.

Abnormalities in the sinus rhythms may be associated with a pathological (a) increase in heart rate (over 100/min) called tachycardias or tachyarrhythmias or (b) reduction in heart rate (less than 60/min) and called bradycardias or bradyarrhythmias. Tachycardias manifest themselves in any one of the following arrhythmias; premature beats, ventricular tachycardias, atrial or ventricular flutter, atrial or ventricular fibrillation (Guyton and Hall, 2000).

### 2.7.1- Techniques used for the production of experimental arrhythmias

In experimentally induced arrhythmias, the techniques used for the production of the arrhythmias may be divided into three groups, namely; chemical, electrical and mechanical (Szekeres 1971, Wilson, 1984). These three differ in the arrhythmogenic stimuli that are used to induce the arrhythmias. Since different arrhythmogenic stimuli exert their arrhythmogenic effect by different mechanisms, it is essential to determine antiarrhythmic activity of any test drug based on arrhythmias induced by different arrhythmogenic techniques.

Antiarrhythmic action may be studied on isolated parts of the heart like the isolated atria and isolated papillary muscle or on the isolated heart using the Langendorff heart preparation and heart-lung preparation, and lastly in the intact animal that is either anaesthetised or unanaesthetised. The rat is particularly suitable for assays where a large number of experimental animals are to be used. The Lambeth Conventions (Walker *et al.*, 1988) give guidelines as well as classification, quantification and analysis of arrhythmias during experimental studies.

### 2.7.1.1 Chemically induced arrhythmias

Various substances with different chemical structure and biological actions have and are used to produce experimental arrhythmias. These chemical agents can be used either alone or in combination. Their arrhythmogenic effect is due to their influence on either the properties of the myocardium or their direct excitation of the central nervous system. Thus, the site of action of these substances is different from one kind of substance to another. The agents are either applied locally directly to the myocardium or injected into the bloodstream.

In local application approach, the drugs act as permanent stimulus at the site of application giving rise to ectopic activity (Szekeres, 1971). The best-known agent is aconitine and it has been used to produce tachycardia, flutter and fibrillation, depending on concentration. Aconitine induces arrhythmia by modifying sodium channels of the cardiac fibres resulting to high –frequency automaticity based on delayed repolarisation (Vaille *et al.*, 1992). Aconitine-induced arrhythmias respond to classes I-III antiarrhythmic drugs. Other drugs that can be used to induce such arrhythmias are acetylcholine, veratrum alkaloids and high doses of cardiac glycosides.

Although arrhythmia can be induced by local application of the agent directly onto the myocardium, the widely used method of chemically inducing arrhythmia is by intravenous application of the arrhythmogenic agent. The agents that are commonly used in this approach include, Barium chloride ($BaCl_2$), calcium chloride ($CaCl_2$), epinephrine, and to some extent chloroform.

#### *2.7.1.1a Induction of arrhythmia by $BaCl_2$*

In mammalian heart, injection of $BaCl_2$ may induce arrhythmias by decreasing the efflux of potassium. According to Szekeres (1971), the decreased potassium efflux enhances rhythmicity of the myocardium and causes prolongation of the action potential. This model is sometimes not preferred because it has a low margin of safety as only slightly higher doses than the one required to induce ventricular extrasystole precipitates ventricular fibrillation (Papp *et al.*, 1967).

#### *2.7.1.1b Induction of arrhythmia by epinephrine*

Injection of epinephrine in higher doses induces arrhythmias that can be readily antagonised by β-receptor blocking agents and other antiarrhythmic agents. Its action lasts for a very short time and according to Szekeres (1971), the effective period is not longer than 30 seconds after its administration.

#### *2.7.1.1c Induction of arrhythmia by $CaCl_2$*

Injection of $CaCl_2$ to the intact animal induces ventricular arrhythmia that eventually leads to fibrillation attributed to direct action of it on the myocardium. $CaCl_2$ is thought to enhance spontaneous ectopic activity and it initiates asynchronous propagation of impulses (Szekeres, 1971). It is advisable to inject the drug very slowly since rapid injection leads to lasting fibrillation resulting in death. $CaCl_2$ perfusion causes ventricular fibrillation within the first minute following its administration. The arrhythmia is due to an increase in the free intracellular $Ca^{2+}$ concentration that promotes abnormal generation of impulses and

alters normal automaticity thereby inducing abnormalities of impulse conduction responsible for re-entry, re-excitation resulting in ventricular fibrillation (Vaille *et al.*, 1992). The authors showed that this model is selective since it is only prevented by agents that block slow inward $Ca^{2+}$ current. These agents belong to class IV antiarrhythmic agents as per Vaughan Williams classification.

#### *2.7.1.1d Induction of arrhythmia by chloroform*

This model has the features of simplicity and economic. The major disadvantages with it are that of being heavily influenced by elevated sympathetic tone and its lack of discrimination when it comes to the classes of antiarrhythmic drugs. Brooks *et al.*, (1989), assert that its lack of discrimination is its major defect since any bradycardia agent, central nervous system and respiratory depressants, and α-adrenolytic agents appear to be active against it. Thus, this model is sensitive to agents belonging to all classes of antiarrhythmic drugs as per Vaughan-Williams classification. On the other hand, it is my opinion that this model might be used as a quick way of checking whether a test compound has potential antiarrhythmic properties since the model is responsive to all classes of antiarrhythmic drugs.

### 2.7.1.2 Mechanically induced arrhythmias

The interventions that induce arrhythmia mechanically alter fundamental properties of the myocardium within a circumscribed area giving rise to local blocks and spontaneously firing ectopic foci. Two models that utilise this approach are the coronary artery ligation model and the coronary artery ligation-reperfusion model. Regional myocardial ischaemia and

reperfusion are both powerful arrhythmogenic stimuli. These arrhythmias are attributed to re-entry and are inhibited by sodium conductance inhibitors and potassium inhibitors (Curtis and Hearse, 1989). Theoretically, both classes of drugs may encourage ventricular fibrillation and ventricular tachycardia to terminate since they effectively increase the re-entry wavelength.

The rat heart is used frequently to investigate arrhythmias resulting from ischaemia and reperfusion because of its lack of collaterals (Maxwell *et al.*, 1987), and because the rat atria exclusively receive its blood supply from extracoronary vessels (Halpern, 1957). This leads to reproducible zones of severe ischaemia upon ligation of the coronary artery. Furthermore, the use of the rats makes much economical sense since large numbers of animals are used in these experiments to generate quantitative information. It is worth noting that the differences observed in rats from other larger hearts compels an investigator to confirm the findings in other species.

The mechanism of initiation of ischaemia-induced arrhythmias is different from the mechanism responsible for initiating the reperfusion-induced arrhythmias. This was shown by Curtis and Hearse (1989), who by using perfused rat heart showed that perfusion of the non-ischaemic tissue with high potassium concentration prevented the induction of ischaemia-induced ventricular fibrillation but not reperfusion-induced ventricular fibrillation. On the other hand, the authors showed that both types of arrhythmias are maintained by a common electrophysiological mechanism since a high intracellular potassium concentration increased spontaneous termination of ventricular fibrillation regardless of whether the fibrillation was induced by ischaemia or reperfusion.

### *2.7.1.2a Coronary artery ligation (CAL) Model*

There are a number of cardiac diseases that involve acute ischaemia and as a result a number of experimental models have been designed to mimic part of the complex events occurring in the human disease. Experimentally, ischaemia is often induced by the occlusion of the coronary artery. In exposed hearts coronary occlusion can be accomplished by typing a ligature around the coronary artery or by clamping. In anaesthetised animals with the chest closed, a coronary artery can be occluded either by inflating a balloon occluder positioned around the artery or by suddenly tightening a snare previously placed around the vessel (Wit and Janse, 1992). Large animals like dogs, pigs and cats are used to study ventricular arrhythmias whilst rats are mostly used to study changes in the metabolic pathways resulting from ischaemic and reperfusion (Janse *et al.*, 1998). The arrhythmias produced by coronary artery ligation are etiologically similar to the arrhythmias occurring in man in association with local ischaemia brought about by heart infarction.

It is thought that there is species difference when it comes to these arrhythmias. These differences are due to differences in the electrophysiological properties of the heart and differences in cardiac and coronary artery anatomy (Wit and Janse, 1992). The variability in the presence or absence of collaterals in different species is one contributing factor to the observed species difference. For instance, pigs and rats are devoid of coronary collaterals, which is not the case with dogs (Maxwell *et al.*, 1987). Thus following coronary occlusion, there is a higher chance of residual blood flow through collaterals in dogs and neither in rats nor pigs. In the larger animals on the one hand, the arrhythmias induced by ischaemia occur in two distinct phases that are commonly called phase 1A and phase 1B (Janse *et al.*,

1998). These phases correspond to the two phases that have been observed in humans. According to Wit and Janse (1992), available data from humans suggest that there is an early phase of acute arrhythmia and a delayed phase of subacute arrhythmia that are separated by a period of decreased arrhythmias. On the other hand, in smaller species like rats there seem to be no clear separation of type 1A from type 1B.

The factors that will determine whether or not this arrhythmia occurs are; the size of the ischaemic area, the degree of collateral flow, heart rate, the mode of coronary artery occlusion, presence of a previous infarction, activity of the autonomous nervous system and hypertrophy in the non-ischaemic myocardium (Wit and Janse, 1992; Janse *et al.*, 1998). These arrhythmias respond best to strong class I agents (Brooks *et al*, 1989). The authors contend that other workers have also shown that class IV agents like Verapamil and nifedipine do suppress extrasystoles that occur in this model.

#### *2.7.1.2b Coronary Artery Ligation/ Reperfusion (CALR) Model*

In experimental animals, restoration of blood flow to the myocardium after a brief period of ischaemia is associated with the occurrence of severe ventricular arrhythmias. This reperfusion-induced arrhythmia is related to the ventricular fibrillation and sudden cardiac death that are seen in humans upon release of coronary spasm or platelet disaggregation causing restoration of blood flow to a previously ischaemic region of the myocardium. Thus, reperfusion arrhythmias are distinct from ischaemia-induced arrhythmias and they are encountered in man (Manning and Hearse, 1984). Unlike the arrhythmias that occur upon coronary artery occlusion, the reperfusion-induced ones have a rapid onset (within 20 seconds after release of ligature) and are of short duration, 1-2 minutes. Their severity is

dependent upon the duration of the preceding occlusion. Kane *et al.*, (1984) showed that the most severe reperfusion-induced arrhythmias occur after an occlusion period of 5 or 15 minutes when 56 and 50 % of the animals developed ventricular fibrillation respectively. The authors showed that when duration of occlusion was reduced to 2 minutes or extended to 20 minutes, the number of the arrhythmias was markedly reduced and no ventricular fibrillation occurred. Thus, the severity of these arrhythmias is highly dependent on the duration of the ischaemia.

The actual mechanisms of initiation and maintenance of reperfusion-induced arrhythmias are less well established and it is unclear whether re-entry or automaticity predominates. The suggestion that abnormal automaticity might be the cause of the arrhythmias is supported by the fact that reperfusion elicits delayed after-depolarisation capable of triggering automaticity (Opie *et al.*, 1986; Pogwizd and Corr, 1986). There is also evidence that suggest that generation of oxygen radicals may play a major role in causing the tissue injury that is associated with reperfusion (Jeroudi, *et al.*, 1994: Roth, *et al.*, 1997).

These arrhythmias, in contrast to those that occur when the occlusion is present, do not appear to be influenced by alterations in autonomic nervous activity. This fact is backed by evidence that has shown that reperfusion-induced arrhythmias are not affected by bilateral vagotomy and $\beta_1$-adrenoceptor blockade with atenolol (Kane *et al.*, 1984). Drugs that block the fast inward sodium channel (class I antiarrhythmic drugs according to the Vaughan Williams classification) are effective, particularly in reducing the incidence of reperfusion-induced ventricular fibrillation in rats. Although class I drugs are generally effective in the rat, they are not clearly antiarrhythmic in dog and pig reperfusion arrhythmia models (Brooks *et al.*, 1989). This might be due to species difference as has been discussed before.

It is also postulated that antioxidants and calcium antagonists may offer a protection against reperfusion injury that results from the increased generation of reactive oxygen radicals (Bernier and Hearse, 1988; Jeroudi, *et al.*, 1994; Roth, *et al.*, 1997). Roth et al., (1997) showed that, during reperfusion, diminished superoxide radical production of circulating neutrophils has beneficial effects on tissue injury that is caused by reactive free radicals. Thus, antioxidants offer a cardioprotection effect during heart ischaemia-reperfusion.

#### 2.7.1.3 Electrically induced arrhythmias

This is the oldest and widely used technique for producing experimental arrhythmias. It has the advantages of not being harmful to the myocardium when the stimuli is not extremely strong, and it can be used to reproduce some of the main types of arrhythmias of clinical importance (Szekeres, 1971). Either a single electrical shock or serial shocks, or progressively increasing rate of stimulation or prolonged application of galvanic current may be performed to produce arrhythmia or fibrillation by electrical stimulation of the heart. According to Szekeres (1971), serial shock is the best method of stimulating the myocardium since with it, it is possible to produce some of the main types of arrhythmias of clinical importance. However, this technique is highly involving and needs expertise and thus would not be recommended for screening anti-arrhythmic drugs but rather it can be used to study those test drugs that have shown a high potential after being screened using the chemically induced arrhythmias.

### 2.7.2 Classification of antiarrhyhmic drugs

Drugs used to restore disorders of cardiac rhythm and rate are known as anti-arrhythmic (or antidysrhythmic) drugs and their pharmacological properties are complex. Antiarrhythmic drugs exert their effects widely by modulating conduction velocity or/and refractory period duration (Janse *et al.*, 1998). Conduction velocity on the one hand, depends on the passive electrical properties of the myocardium, and on the characteristics of the sodium ($Na^+$) and $Ca^+$ channels. On the other hand, potassium ($K^+$) currents determine repolarisation that in turn determines the action potential duration and the refractory period duration. According to Janse *et al.*, (1998), agents that prolong the action potential duration, and thereby the refractory period, are effective against re-entrant arrhythmias by prolonging the wavelength. The prolongation of the wavelength either prevents the initiation of a re-entrant arrhythmia by a premature impulse or terminates an existing arrhythmia.

There are various ways of classifying anti-arrhythmic drugs, but the most widely accepted classification is the one suggested by Vaughan Williams (1991). This classification is based on the action of the drug on the electrophysiological properties of the myocardium tissue and on the interaction with membrane receptors and ion channels. This classification is by no means rigid as some drugs have effects belonging to more than one class. An example of this is Quinidine that has both class I and class III properties. In addition, many of the anti-arrhythmic drugs have other clinical uses such as vasodilators, antihypertensive, anti-cholinesterase.

### 2.7.2.1 Class I anti-arrhythmic drugs

These drugs directly change membrane conductance of ions, particularly those of $Na^+$ and $K^+$. Thus, these drugs exert their antiarrhythmic effect by depressing the conduction velocity, prolonging the refractory period and increasing the threshold of excitability of the myocardium (Vogel and Vogel, 1997). Depending on the effect of the drugs on the action potential duration, class I drugs are further subdivided into classes IA, IB and IC.

Class IA drugs lengthen the action potential duration that is reflected on the ECG reading as lengthening of the QT-interval. Examples of drugs belonging to this class are Quinidine, disopyramide and procainamide. Class IB drugs shorten the action potential duration and examples of the drugs in this class are lodoacaine, phenytoin and mexiletine. Lastly, class IC drugs produce effects of both class IA and class IB drugs (Vogel and Vogel, 1997).

### 2.7.2.2 Class II anti-arrhythmic drugs

These drugs reduce adrenergic influence on the heart. These β-adrenergic antagonists exert their anti-arrhythmic effects by either reducing or blocking the electrophysiological effects of catecholamines that are mostly mediated by an increase in the slow $Ca^{2+}$ inward current (Vogel and Vogel, 1997). Examples of drugs belonging to this class are atenolol, timolol and bretylium.

#### 2.7.2.3 Class III anti-arrhythmic drugs

Drugs belonging to this class prolong the action potential and thus lead to an increase in the effective refractory period. Blocking of the outward repolarising currents is the main mechanism of action of these drugs (Vogel and Vogel, 1997).

#### 2.7.2.4 Class IV anti-arrhythmic drugs

Class IV agents block slow $Ca^{2+}$ channels thereby suppressing the slow $Ca^{2+}$ inward current and calcium dependent slow action potentials (Vaille, *et al.*, 1992; Vogel and Vogel, 1997).

## 2.8- PHARMACOLOGY OF INOTROPIC DRUGS

Inotropic mechanisms effectively influence contractility of muscular tissue such as the heart. In the heart, positive inotropic (cardiotonic) interventions strengthen the force of contraction of the heart whilst negative inotropic interventions weaken muscular contraction (Scholz, 1984; Siegl, 1986). The cardiac contractile state in turn determines how well the heart functions as a pump by muscle shortening, developing pressure in the ventricular cavity, and ejecting blood. The increase in the contractility of the heart is ultimately dependant on either the increase in the intracellular free $Ca^{2+}$ that interacts with the contractile proteins or to an increased sensitivity of the myofilaments for the $Ca^{2+}$ or both.

Inotropic drugs are currently used to preserve or restore myocardial function in patients with heart disease. Because depression of the myocardial contractility plays a vital role in the development of heart failure, it is logical to attempt to enhance the inotropic state of the failing heart by using positive inotropic agents. Thus, efforts have been directed at developing positive inotropic agents that increase the contractile performance of the heart without elevating myocardial oxygen consumption (i.e. increasing heart rate and vascular resistance) or evoking arrhythmias. In hypertrophic cardiomyopathy, negative inotropic interventions are used to improve cardiovascular hemodynamics (Siegl, 1986; Sasayama, 1996).

### 2.8.1 Regulation of cardiac muscle contraction and relaxation

Depolarisation of the sarcolemma, that is the sodium ($Na^+$)- dependent upstroke of the action potential, is the initial event. This depolarisation of the cell membranes allow the extracellular $Ca^{2+}$ to move down its electrochemical gradient into the cell through membrane bound $Ca^{2+}$ channels. This $Ca^{2+}$ (slow $Ca^{2+}$ inward current) serves to fill intracellular stores and triggers the release of much larger amounts of activator $Ca^{2+}$ from the sarcoplasmic reticulum into the cytosol where it reacts with the contractile apparatus. Interaction of the activator $Ca^{2+}$ with troponin C initiates molecular re-arrangement of regulatory proteins, tropomyocin and troponin, thereby allowing interaction of actin and myosin. Because of crossbridge attachments between actin and myosin, cardiac muscle contracts, and tension is developed. Dissociation of $Ca^{2+}$ from the contractile apparatus and $Ca^{2+}$ sequestration by the sarcoplasmic reticulum leads to relaxation. Cyclic adenosine monophosphate (AMP) modulates the handling of calcium in the sarcoplasm through

activation of a variety of cyclic AMP- dependent protein kinases. This is done by catalysing the transfer of the terminal phosphate of adenosine triphosphate (ATP) to form phosphoesters with several functional proteins within the cell in process termed phosphorylation. (Scholz, 1984; Siegl, 1986; Sasayama, 1996).

All inotropic drugs interact with one or more of the above steps involved in the contraction and relaxation of the cardiac muscle. The positive inotropic drugs bring about their effects either by increasing cyclic AMP in the cytosol or by increasing the free intracellular $Ca^{2+}$ and the sensitivity for the available $Ca^{2+}$.

#### 2.8.1.1 Cyclic AMP increasing agents

Agents that increase the concentration of intracellular cyclic AMP are expected to enhance the inotropic state of the heart by increasing the cytosolic calcium. Intracellular cyclic AMP can be increased either by promoting its synthesis by the activation of adenylate cyclase or by inhibiting its degradation using phosphodiesterase inhibitors. The major disadvantage with drugs belonging to this class is that increased cyclic AMP is linked to potential arrhythmogenic effects and increased energy expenditure.

##### 2.8.1.1a *Activation of adenylate cyclase*

Adenylate cyclase is an enzyme that is located on the intracellular surface of the sarcolemmal membrane and it catalyses the conversion of ATP to cyclic AMP. Adenylate cyclase can be activated either by stimulation of receptors coupled to it or by a direct action on the enzyme itself or by a subunit of the enzyme.

There is a lot of evidence that suggest that agonists of the following sarcolemmal membrane receptor systems activate adenylate cyclase; β-adrenergic, $H_2$– histaminergic and glucagons. Agonists of β-adrenergic receptors are the most widely used activators of adenylate cyclase, and they have been shown to have positive inotropic effect. Examples of these β-adrenergic agonists are norepinephrine, epinephrine, isoproterenol, dopamine and dobutamine. The major problem with drugs that use this mechanism is that there is a possibility of the development of tolerance at the receptor site resulting in the reduction of the inotropic effect.

Apart from indirect activation through the receptors, adenylate cyclase can also be activated directly. The best example that has been shown to work in this way is forskolin, a derivative from a medicinal plant called *Coleus forskohlii*. Forskolin stimulates adenylate cyclase in membranes and intact cells (Seamon *et al.*, 1981). It has been shown to have a positive inotropic and chronotropic effects, and a blood pressure lowering activity (Lindner *et al*, 1978; Metzger *et al*, 1981). It also stimulates the voltage-sensitive calcium current that is believed to play a role in the inotropic effect (Hartzell and Fischmeister, 1987). The positive inotropic effect of forskolin is very similar to that induced by β-adrenergic agonists although the former does not require neither membrane surface receptors nor a functional guanine nucleotide-binding subunit (Seamon and Daly, 1981). Nevertheless, it is believed that forskolin requires specific binding site to induce its cardiac activities since derivatives of it with some modifications fail to have any effect on isolated heart preparations nor in activating adenylate cyclase (Seamon *et al.*, 1983; Schmidt and Kukovetz, 1989).

#### 2.8.1.1b *Inhibition of phosphodiesterase*

Intracellular cyclic AMP concentration can also be increased by reducing its degradation to inactive metabolites by phosphodiesterase (PDE). Inhibition of PDE activity elevates the concentration of cyclic AMP and thereby enhancing developed tension. Examples of drugs that belong to this class of positive inotropic drugs are amrinone and 1-methyl-3-isobutylxanthine (IBMX) (Sasayama, 1996; Xiong *et al.*, 2001). Phosphodiesterase inhibitors have an advantage over β-adrenergic agonists because their ability to increase cyclic AMP concentration is not diminished by membrane receptor tolerance. Thus, these drugs are suitable for patients with congestive heart failure who require chronic inotropic therapy.

### 2.8.1.2 Drugs that increase either free-intracellular $Ca^{2+}$ or $Ca^{2+}$ sensitivity

As mentioned before, the increase in force of myocardial contraction is ultimately due to an increase in intracellular free calcium that interacts with the contractile apparatus or to an increased sensitivity of the myofilaments for $Ca^{2+}$. Inhibitors of $Na^{+}$,$K^{+}$-ATPase, like digitalis glycosides, belong to the class of drugs that increase intracellular free calcium. It is thought that inhibitors of $Na^{+}$,$K^{+}$-ATPase cause an increase in the intracellular sodium ion concentration. This elevated intracellular sodium activity enhances $Ca^{2+}$ entry and/or reduces $Ca^{2+}$ efflux via sodium-calcium exchanger system. Thus, there is an increase in the development of cardiac tension because of the increased concentration of intracellular free calcium.

Modulating the sensitivity of the contractile apparatus, especially the troponin C, for the free $Ca^{2+}$, can also regulate contractility of the cardiac muscle. For instance, magnesium ions and acidic pH have been shown to decrease the sensitivity of contractile proteins for $Ca^{2+}$ (Ogawa, 1985). On the other hand, several calmodulin inhibitors like bepridil, calmidazolium and sulmazole, increase the sensitivity of contractile proteins to calcium (Herzing *et al.*, 1981; Silver *et al.*, 1986; Herzing *et al.*, 1996). These calcium sensitising agents would be vital in patients who are in a state of lack of activator calcium as it happens in hypocalcaemia. The only problem would come when these agents are applied in a state of increased activator calcium as present in ischaemic myocardium (Francis and Cohn, 1990). There are however, reports of some extracts that have a safe inotropic principle able to work under both low and high calcium load. Gilani, *et al.*, (1999) have reported of a n-butanolic fraction from *Berberis aristata* fruit which might be cardiotonic under conditions of low levels of activator calcium and cardioprotective under ischaemic calcium overload.

## 2.9- MEDICINAL PLANTS

For a very long time plant and animal products with therapeutic values have been used as the main source of drugs. Despite the increase in the use of synthetic drugs, plants remain one of the major sources of drugs. Rates (2001) asserts that of the 252 drugs considered as basic and essential by the World Health Organisation (WHO), 11 % of these are exclusively of plant origin and a significant number of the remainder are synthetic drugs obtained from natural precursors. Examples of drugs obtained from plants are; digoxin, quinine, quinidine, vincristine, vinblastine, atropine and penicillin.

Apart from direct use of plant materials, plants also provide lead compounds that have and still are leading to the discovery of new therapeutic properties (Hamburger and Hostettmann, 1991). The newly discovered therapeutic properties are then used in designing and synthesizing new drugs with similar or more potent therapeutic values. Lastly, plants provide compounds that are used as experimental tools in pharmacological, biochemical, haematological and physiological studies. Examples of such compounds are forskolin, cannabinoids, muscarine, yohimbine and colchicines (Williamson *et al.*, 1996).

The interest in the use of natural products derived from plants for therapeutic purposes has seen a tremendous increase in demand recently. It is estimated that about 80% of the world population relies on medicinal plants for their primary health care needs (Vulto and Smet, 1988; Groombridge, 1992; WHO/IUCN/WWF, 1993). This demand in natural products has seen a rise in the world market for it, and according to Soldati (1997) the world market for over the counter phytomedicinal products was worthy more than US $ 10 billion. The sales of these products are increasing at the rate of about 10 % each year in most developed countries (Houghton and Raman, 1998) and in South Africa the market is estimated to be worth R2.3 billion per year (Copeland, 2003). The interest in natural products has also led to the WHO to declare 31$^{st}$ August as the day of African tradition medicine with 2000-2010 being declared as the decade for African tradition medicine (WHO, 2003). Furthermore, to show the importance of natural products, WHO and most governments consider phytotherapy in their health programs and they have set up basic procedures to be followed when validating drugs that are sourced from plants (Vulto and Smet, 1998). In South Africa, the Medicines Control Council has established a unit dealing with complementary medicine, and has earmarked more than 10,000 substances for evaluation and regulation (Copeland, 2003).

Plants can be used as therapeutic resources in three main ways. They can be used firstly as herbal teas or home made remedies, secondly as crude extracts and thirdly as isolated active compounds after been subjected to successive extraction and purification processes (Rates, 2001). When used as herbal teas or other home made remedies it is with the assumption that the plant is a medicinal one. By definition, a medicinal plant is either any plant used in order to relieve, prevent or cure a disease or alters physiological and pathological process, or any plant employed as a source of drugs or their precursors (Rates, 2001). Plants are used as crude extracts when they are considered as phytopharmacetical preparations or herbal medicines. In this case, the material is obtained exclusively from a plant in the crude state. When pure active compounds are to be used, plants are subjected to successive extraction and purification procedures in order to isolate the compounds of interest. On the one hand, when these compounds are active themselves they can be used directly as drugs examples of which are quinine and digoxin. On the other hand, compounds like diosgenin can be used as precursors in hemisynthetic processes or as models for total synthesis with well-defined pharmacological activity or as models for structure-activity relationship studies used to determine prototype drugs (WHO, 1992; Rates, 2001).

### 2.9.1 Selecting a Plant

A suitable plant is selected as a candidate source for herbal medicine or an isolated active compound in several ways. These include random collection of plant material, targeted collection based on consideration of chemotaxonomical relationships, observation of tradition use of plants in folk medicine, observation of plant toxic effect to animals and its

surrounding, and a combination of several criteria (Malone, 1983; Soejarto, 1996; Williamson *et al.*, 1996).

The strategy widely used in selecting a plant is the careful observation of the use of the plant resources in folk medicine commonly referred to as ethnopharmacology or ethnobotany. The preparation procedures of plants used by ethnic groups provide vital information of how best to extract active compounds from the plant materials. According to Rates (2001), the formulation used in folk medicine provides information about pharmacological activity, the best route of drug administration, and the doses to be tested. It is worthy pointing out that extracts of plants selected using this approach should not just be tested for the pharmacological action claimed by the ethnic people but rather should be screened on a wide range of models as possible (Williamson *et al.*, 1996). It is my view that this approach of screening the extracts on a wider range of models would help in identifying novel therapeutic properties that would otherwise not been known had the investigator stuck to only the tradition use of the plant.

In terms of chemical content, once a therapeutic action of a particular plant has been attributed to a certain compound, it is possible to hypothesise that plants that contain similar compounds would have similar therapeutic values. According to Williamson *et al.*, (1996), this approach is greatly dependant on chemotaxonomic information relating different classes of compounds to different plant species.

The other plant selection approach that has yielded many vital drugs is by working with plants that are known to indigenous people as being poisonous. Williamson *et al.*, (1996) assert that poisonous plants more than plants used regularly in folk medicine have a higher

potential to contain highly specific and potent compounds that can be used as drugs or as probes for the elucidation of biological phenomena. Examples of important drugs of plant origin that came from poisonous plants are; tubocurarine (arrow poison), atropine (poison), picrotoxin (fish poison), muscarine (poisonous mushroom), dicoumarol (poisonous clover), and physostigmine (ordeal poison).

Once the plants are selected as possible sources for drug development, it is vital to take into account the preservation of natural diversity and environment. The preservation prevents the extinction of some plant species that may have interesting chemical compounds for potential drug development or may have genes vital for the biosynthesis of new compounds (Soejarto, 1996; Brito and Nunes, 1997; Rouhi, 1997). Thus, there is need for the encouragement of plant preservation and replanting, and sensible use of the plant resources in terms of the amount and parts of the plant. People should be encouraged to use parts that can easily regenerate without killing the plant, for instance leaves and barks should be used more than using roots.

### 2.9.2 Preparation of the plant material and isolation of active compounds

The process of getting a pure biologically active compound is complex and involves a multidisciplinary collaboration of botanists, plant taxonomists, pharmacognosists, chemists, pharmacologists and toxicologists (Hamburger and Hostettmann, 1991). Once a plant is selected for drug development, there is need for a botanist to taxonomically identify it and collect part of the plant for herbarium preservation. This "voucher specimen" is deposited in a herbarium and it serves as a reference material for future use. It is common practice to record the date, time, season, location and terrain from where the material was collected.

Once this is done, the collected material is subjected to a stabilisation process that is usually done by drying at ambient temperature under a shady area. In some studies, the materials are dried in an oven with controlled air flow (Conde Garcia *et al.*, 2003). In cases where the stability of the test compound is unknown, the stabilisation process involves freezing, lypophilisation and use of alcohol vapour (Williamson *et al.*, 1996; Houghton and Raman, 1998).

The dried material is cut into very small pieces and if possible ground into powder before being subjected to a suitable extraction process to yield crude extracts. The extraction process involves mostly the use of solvents with varying polarity. Water is also used as a solvent since in traditional practices it is the solvent of choice. If the compound of interest is known, the selected method of extraction is the one that gives the highest yield and purity of the compound. Preliminary chromatographic analysis by thin layer chromatography (TLC) and/or high-performance liquid chromatography (HPLC) is then done to get an idea on the kind of compounds available in the crude plant extract (Hamburger and Hostettmann, 1991).

The crude extracts are subjected to bioassay and toxicity evaluation to check if they have any biological activity. Then, detailed chromatographic methods like TLC and column chromatography are employed to obtain isolated active compounds. To get pure compounds, it is imperative that the active plant extracts are sequentially fractionated and then each fraction obtained is submitted to bioassays and toxicity evaluation (Houghton and Raman, 1998; Rates, 2001). The easiest toxicity evaluation can be performed using brine shrimp toxicity test (Meyer *et al.*, 1982). The brine shrimp test is easy, cheap and rapid, and thus convenient as a general bioassay tool.

The next step is to verify the purity of the isolated compounds before being submitted for the determination of their chemical structure. The elucidation of the structure can be done by spectroscopic methods like; ultraviolet/visible spectrum, infrared (IR) spectrum, mass spectrum and nuclear magnetic resonance (NMR) spectra (Houghton and Raman, 1998).

The last steps involve partial or total synthesis of the compounds. It is also vital to prepare derivatives or analogues of the compounds for the investigation of structure-activity relationships. In most cases, the derivatives are bound to have a different action from the original compound. Lastly, if the compounds have a high commercial value, a large–scale isolation is commissioned for further pharmacological and toxicological tests.

## 2.10 BOTANY OF PLANTS USED IN THIS STUDY

The medicinal plants that were used in this study were selected based on their reported triterpenoids content according Anne Hutchings Inventory of Zulu Medicine (1996). This strategy involved targeted collection of plants based on consideration of chemotaxonomical information that was found in the above inventory. The following sections describe the botany of the medicinal plants that were used in this study.

### 2.10.1 *Buddleja salviifolia* (LOGANIACEAE)

This plant is found in various tropical countries. This shrub can grow up to five meter in height and has a spread of 4 meters. It produces silver grey powdery leaves and white to cream or lilac to purple coloured flowers (Figure 1)

Figure 1. *Buddleja salviifolia* shrub (www.images.google.com)

In tradition medicine, the roots have been reported to be used as decoctions used for stomach upsets, flatulence, and diarrhoea and as a gargle for coughs. Leaves are used as an eyewash and for colic whilst flower decoctions are used to watch sores (Watt and Breyer-Brandwijk, 1962; Roberts, 1990). Verbascoside has been isolated from this plant

### 2.10.2 *Psidium guajava* (MYRTACEAE)

*Psidium guajava*, commonly called guava, occurs naturally in Central America but is found in many parts of the world where it has become naturalised. It is a shrub or a small tree that has a characteristically smooth trunk that result from the peeling off the barks. It produces small white flowers in summer, followed by rounded or pear-shaped, many-seeded fruit (Figure 2). The inside of the fruit can be pink, white, or red.

Guava leaves are commonly used for diarrhoea (Watt and Breyer-Brandwijk, 1962; Hutchings *et al.*, 1996; Van Wyk *et al.*, 1997). The leaves are also used for other ailments including toothache, diabetes, fever, coughs, ulcers, boils and wounds (Van Wyk *et al.*, 1997; Jaiar *et al.*, 1999; Tona *et al.*, 1999). Roots are used for venereal diseases, leprosy,

abdominal pains and scabies (Hutchings *et al.*, 1996). In Taiwan, fruits are used to treat diabetes mellitus (Hsu and Cheng, 1992).

Figure 2. Leaves and fruits of *Psidium guajava* (www.images.google.com)

The chemical constituents found in the plant include essential oils, triterpenoids like cratergolic, guaijavolic, oleanolic and ursolic acids), and numerous tannins (Osmna *et al.*, 1974; Smith and Siwatibau, 1975; Morton, 1981; Lutterodt, 1989). Ellagic acid found in the leaves is thought to contribute to the beneficial effects observed in its use in treating diarrhoea since the acid is a known astringent and haemostatic (Bruneton, 1995). Antibiotic activity is attributed to guajaverin and psidolic acids (Bārdy *et al.*, 1982). Quercetin is thought to contribute to the anti-oxidant, anti-carcinogenic, anti-HIV and antibiotic effects observed with the plant (Cheng and Yang, 1983; Loyoza *et al.*, 1994; Van Wyk *et al.*, 1997). Fruit extracts lower blood glucose levels in streptozocin-induced diabetic rats (Hsu and Cheng, 1992). Guava leaves have also been shown to have antinociceptive effects in chemical and thermal tests of analgesia (Lutterodt and Maleque, 1988; Re *et al.*, 1999; Shaheen *et al.*, 2000).

### 2.10.3 *Syzygium cordatum* (MYRTACEAE)

*S. cordatum* is found in the tropical regions, and in South Africa it is widely distributed in the eastern and north-eastern parts. The tree is medium-sized and grows up to 15 meters in height. The plant has rough dark brown bark and the leaves are broad, with a bluish green colour (Van Wky, *et al.*, 1997). It produces cream- to pinkish coloured flowers that give way to egg-shaped, red to dark purple berries (Figure 3).

The plant is used to treat respiratory ailments, tuberculosis, stomach complaints and diarrhoea (Watt and Breyer-Brandwijk, 1962; Iwu, 1993; Hutchings *et al.*, 1996). The bark and roots are used for headaches and wounds whilst the root on its on is used to increase the flow of milk in nursing mothers (Hutchings *et al.*, 1996).

Figure 3. *Syzygium cordatum* (www.images.google.com)

Compounds isolated from wood and bark include pentacyclic triterpenoids such as friedelin, arjunolic acid, gallic acid, and steroidal triterpenoids such as β-sitosterol and

ellagic acid (Candy *et al.*, 1968; Bruneton, 1995). The exact biological activity of this plant is not well known but the effects are most likely to be those associated with triterpenoids. Triterpenoids have been shown to have a wide variety of biological activities as has already been discussed in chapter one of this thesis.

### *2.3.4 Centella asiatica* (APIACEAE)

*C. asiatica* is found in Africa, Asia, Australia, South America and the southern part of North America. (Van Wyk *et al.*, 1997). It is a perennial weed that is often found in moist places. This creeping plant forms thin stems with characteristically round or kidney-shaped leaves on long, slender stalks (Figure 4).

Figure 4. *Centella asiatica* leaves (www.images.google.com)

The plant is reported to be used for treating leprosy and various skin conditions, and it is no wonder that it said to be a vital constituent of dermatological products (Bruneton, 1995). It

is also widely used for wound treatment, fever, syphilis and as a diuretic and purgative (Iwu, 1993). Cheng and Koo (2000) presented results that suggested that *Centella asiatica* prevented ethanol induced gastric mucosal lesions by strengthening the mucosal barrier and reducing the damaging effects of free radicals. The wound healing activity is attributed to a triterpenoids saponin called asiaticoside (Van Wyk *et al.*, 1997; Brinkhaus, *et al.*, 2000). It is also reported that asiaticoside and other triterpenoids available in the plant have anti-tumour properties (Babu *et al.*, 1995). Total triterpene fraction of *Centella asiatica* (TTFCA) has been shown to improve microcirculation and capillary permeability in patients with venous hypertension (Belcaro *et al.*, 1990a; Belcaro *et al.*, 1990b; Cesarone, *et al.*, 1994). The effect of the plant on microcirculation and capillary permeability might explain why the plant is used for the treatment of varicose veins and haemorrhoids (MacKay, 2001). In Chinese medicine the plant is used to alleviate symptoms of depression and anxiety due to its anxiolytic activity (Bradwejn *et al.*, 2001). Other biological activities attributed to *C. asiatica* include antibacterial, antifungal, anti-inflammatory, anti-allergic, hypotensive, antipyretic and peptic ulcer healing (Van Wyk *et al.*, 1997).

### *2.3.5 Prunus africana* (ROSACEAE)

*Prunus africana* is found mainly in afromontane forests along the mist belt regions of South Africa and further north into tropical Africa (Von Breitenbach, 1986). It is a tall tree that may reach more than 30 meters in height (Figure 5). The tree has buttress roots and a coarse, dark brown and black coloured bark. The dark green, glossy leaves smell of almonds when crushed. The tree produces small white flowers that are followed by reddish-brown berries (Coates Palgrave, 1977; Schippmann, 2001).

The bark is used to treat intercostal pain (Pujol, 1990) and benign prostate hypertrophy

Figure 5 a and b. *Prunus africana* tree (www.images.google.com)

(Cunningham and Mbenkum, 1993; Bruneton, 1995). A water extract of the stem-bark is traditionally used in the treatment of bovine basesiosis whilst a powdered stem-bark mixed in water is used traditionally as a remedy for stomachache and as a purgative in humans and animals (Kokwaro, 1976).

Some of the chemical constituents that have been isolated from the plant include phytosterols, pentacyclic triterpenoids esters, amygdalin that is a cyanogenic glycoside (Watt and Breyer-Brandwijk, 1962; Bruneton, 1995). Bark extracts yield friedelin, ursolic acid, maslinic acid, 2α-hydroxyursolic acid, epimaslinic acid (Catlano *et al.*, 1984). The reported activity of the tree against prostatic adenoma is possibly due to β-sitosterol (Debat, 1970). Oral administration of aqueous extract of *Prunus africana* stem-bark in rats has been shown to cause a slight dosage rate-associated increase in plasma alanine

aminotransferase (ALAT) and plasma creatine kinase (Gathumbi *et al.*, 2000). The increase in ALAT suggests that the extract causes liver damage whilst a rise in plasma creatine kinase is indicative of the presence of cardiotoxic components in the plant.

### 2.10.6 *Terminalia sericea* (COMBRETACEAE)

*T. sericea* is found in the sandy savanna areas of Southern Africa (Coates Palgrave, 1977). The tree grows to about 5-8 meters in height and has a wide spreading crown. The coarsely fissured bark is grey to brown in colour whilst the leaves are characteristically silver-haired and crowded at the branch tip (Figure 6 a and b). The tree produces long fruits with two broad papery wings surrounding the thickened central part (Palmer and Pitman, 1972).

Roots are used as remedies for stomach disorders, diarrhoea (Hutchings *et al.*, 1996), treating schistosomiasis, pneumonia and eyewashes (Palgrave, 1981), infertility, venereal diseases, gynaecological disorders, general weakness and sore throats (Gelfand *et al.*, 1985). The bark is used to treat diabetes and wounds (Watt and Breyer-Brandwijk, 1962) whilst the leaves have been reported to be used to treat wounds and menorrhagia (Hutchings *et al.*, 1996).

Figure 6 a and b. *Terminalia sericea* (www.images.google.com)

Several pentacyclic triterpenoids have been isolated from this tree, of which sericic acid and sericoside are the main compounds (Bombardelli *et al.*, 1974). These triterpenoids have been shown to have anti-ulcer, anti-inflammatory and cicatrising activity. The trees also produce tannins, which might explain the anti-diarrhoeal activity attributed to the plant (Hutchings *et al.*, 1996).

### 2.10.7 *Plantago major* (PLANTAGINACEAE)

The plant is thought to have originated in Europe but it is now commonly found naturalised throughout the world (Wells *et al.*, 1986). *Plantago major* ((Figure 7 a and b) together with *Plantago lanceolata* (Figure 8) are cosmopolitan annual or perennial hbs that are often stemless (Hutchings *et al.*, 1996). It can grow up to 15 meters with the size varying depending on the growth habitat. The leaves grow in rosettes, and they are ovate to elliptical with parallel venation. The flowers are small, brownish-green in colour and growing on long non-ramified spikes (Samuelsen, 2000).

Figure 7 a and b. *Plantago major* (www.images.google.com)

Recent ethnopharmacological studies show that this herb is used in many parts of the world to treat a variety of diseases. For instance, the plant decoctions are used for eye infections, toothache, rheumatism and uterine problems, and as vermifuges, tonics and diuretics (Gurib-Fakim *et al.*, 1993). According to Samuelsen (2000), the plant is also used to treat skin diseases, infectious diseases, problems concerning the digestive system, respiratory system, reproduction, the circulation, against tumours, for pain relief and for reducing fever.

The seeds contain a lot carbohydrates and lipids (Samuelsen, 2000). Compounds isolated from the leaves include tannins, oleanolic acid, iridoids, catalpol and flavonoids (Pailer *et al.*, 1969; Hutchings *et al.*, 1996). Extraction work on the leaf wax yielded oleanolic acid, ursolic acid, 18β-glycyrrhetinic acid and sitosterol (Ringbom *et al.*, 1998). Studies in China have shown that the plant extracts have anti-microbial activity and complex cardiovascular effects in animals (Chan and But, 1986). Other biological activities that have been attributed to *P. major* include anti-ulcerogenic activity (Yesilada *et al.*, 1993), antioxidant and free radical scavenger activity

Figure 8 *Plantago lanceolata* (www.images.google.com)

(Campos and Lissi, 1995). The reported diuretic effect of this plant is debatable since whilst some investigators have confirmed it (Cáceres *et al.*, 1987) others have failed to confirm it (Doan *et al.*, 1992). This difference might be due to differences in the species of animals used in the studies since the former group tested the diuretic effect in rats whilst the latter group tested the effect in humans.

### 2.10.8 *Tetradenia riparia* (LAMIACEAE)

*T. riparia* (Figure 9 a and b) is common in the tropical Africa and its distribution extends from eastern parts of South Africa into Namibia and Angola and northwards through east tropical Africa to Ethiopia (Codd, 1985; Hutchings *et al.*, 1996). The plant is a perennial shrublet or soft shrub that has succulent leaves and stems with glandular hairs. The leaves are strongly aromatic.

Figures 9 a and b. *Tetradenia riparia* (www.images.google.com)

Leaf decoctions and infusions are used for respiratory ailments, diarrhoea, influenza, malaria, gall sickness and dropsy of the lower limbs (Hutchings *et al.*, 1996).

Compounds from leaves include the diterpenoid diol and various similar diterpenoids such as 8(14), 15-sandaracopimaradiene-7α, 18 diol, umuravumbolide, ibozol, and deacetylboronolide (Hakizamungu *et al.*, 1988). The diterpenoid diols from the leaves (particularly the 7,18-diol) have antispasmodic activity guinea-pig ileum and on noradrenalin-induced contractions of rabbit aorta (Van Puyvelde *et al.*, 1987). Extracts from leaves have also been shown to have *in vitro* antibiotic activity (Van Puyvelde *et al.*, 1986; Van Puyvelde *et al.*, 1994).

### 2.10.9 *Combretum molle* (COMBRETACEAE)

The *Combretum molle* (Figure 10) is common in the tropics especially in Africa. The tree produces fruits that have four or more wings on it.

Figure 10. *Combretum molle* tree (www.images.google.com)

Fresh or dry leaves are used for dressing wounds, snakebites, fever and anthelmintics, and as abortifacients (Watt and Breyer-Brandwijk, 1962). Roots are used for constipation, swelling of the abdomen, infertility, diarrhoea, bleeding after birth, convulsions, and as an aphrodisiac (Gelfand *et al.*, 1985).

Chemical constituents that have been isolated from the plant include mollic acid, xyloside, arabinoside and large amounts of triterpenoids (Rogers and Thevan, 1986; Lawton and Rogers, 1993). The relative abundance of triterpenoids might explain the anti-inflammatory, ant-microbial and molluscicidal activities associated with this plant.

### 2.10.10 *Eugenia jambolana* (MYRTACEAE)

The plant is found in the tropics and some parts of North America. The tree produces green unripe berries that become purple when ripe (Figure 11).

Figure 11 *Eugenia jambolana* (www.images.google.com)

The pulp extract as well as the seed extract of *E. jambolana* fruits display hypoglycaemic activity although the action is said to be speedy (Achrekar, *et al.*, 1991; Wang and Ng, 1999; Grover, *et al.*, 2000). Oral administration of the extract in normoglycemic and streptozotocin-induced diabetic rats causes the serum insulin levels to rise. It has also been shown that incubation of the extracts with isolated islets of Langerhans from normal and diabetic rats augments insulin secretion from the islets. These extracts inhibited insulinase activity from liver and kidney (Achrekar, *et al.*, 1991; Wang and Ng, 1999; Vikrant, *et al.*, 2001). Although many studies have shown the hypoglycaemic activity of this plant, one study reported that the plant does not exhibit this activity. Pepato *et al.*, (2001) reported that *Eugenia jambolana* leaf decoction had no antidiabetic activity in Streptozotocin-diabetic rats treated for 17 days. This discrepancy might only be explained as being due to differences in the extraction process between the groups. It is my view that extraction processes can result in losses of active compounds

The various plants belonging to *Eugenia uniflora* L. (Myrtaceae) are used in North-eastern Argentina as a treatment for hypertension. Intraperitoneal administration of the aqueous crude extract (ACE) decreased blood pressure of normotensive rats (Consolini, *et al.*, 1999). The results from this work suggest that the empirical use of *Eugenia uniflora* L. (Myrtaceae) is mostly due to a hypotensive effect mediated by a direct vasodilating activity, and to a weak diuretic effect that could be related to an increase in renal blood flow. Although, the authors do not specify the species of Eugenia used in their study, one can postulate that *Eugenia jambulana* might also have its hypotensive through the above-mentioned mechanism.

Various terpenoids including monoterpenoids, sesquiterpenoids, and triterpenoids have been isolated from the plant. The plant also produces saponin. Oleanolic acid isolated from its flowers has been reported to have strong anti-fertility effect in male albino rats (Rajasekaran *et al.*, 1988).

Ethanol extracts of the plant showed significant inhibitory activity against castor oil induced diarrhoea and PGE2 induced enteropooling in rats. The extracts also showed a significant reduction in gastrointestinal motility in charcoal meal tests in rats Thus, the results obtained establish the efficacy of the plant materials as anti-diarrhoeal agents (Mukherjee, *et al.*, 1998)

### 2.10.11 *Clerodendrum trichotomum* (VERBENACEAE)

The plant is found in the warm tropical areas. It is a shrub that produces light to dark-pink flowers (Figure 12).

The plant has been reported to be used to reduce blood pressure. In addition to the blood lowering effect, plants belonging to this genus have been shown to have anthelminitic properties, sedative effects and muscle stimulating action (Gupta *et al.*, 1994)

Figure 12. *Clerodendrum trichotomum* (www.images.google.com)

The chemical constituents that have been isolated from the plant might include saponins. Intravenous administration of extracts from the dried leaves elicited renal vasodilatation and increased urine flow and urinary sodium excretion in dogs and rats (Lu *et al.*, 1994). Oral administration reduced blood pressure of spontaneously hypertensive rats but not normotensive control rats. Chronic administration also prevented increases in blood pressure of spontaneously hypertensive rats.

### 2.10.12 *Psychotria serpens* (RUBIACEAE)

The plant is found in the tropical areas. *P. serpens* is a shrub that has succulent leaves and stems. It has its inflorescence at the terminal and the flowers bud green and turn white (Figure 13). It produces green bellies that turn yellow to orange in colour.

Whole herbs of *Psychotria serpens* are used a lot in folklore medicine. In Taiwanese folklore, the whole herb is used as an antirheumatic, analgesic, muscles relaxing, and circulation promoting drug (Chung, 1979).

Ursolic acid was found to be one of the active principles of cytotoxic anti-leukaemic extracts from dried whole plants (Lee *et al.*, 1988). The authors clearly indicated that ursolic acid showed significant cytotoxicity against the growth of lymphocytic leukaemia cell lines as well as a human lung carcinoma cell line. It also showed marginal cytotoxicity in the human colon and mammary tumour cells.

Figure 13. *Psychotria serpens* (www.images.google.com)

# 3.0 MATERIALS AND METHODS

## 3.1- ETHICAL CONSIDERATION

The Ethic Committee of the University of Durban-Westville approved the procedures followed in this study. Principles of laboratory animal care were followed.

## 3.2 MATERIALS

Silica gel, TLC plates and all the solvents were purchased from Merck, SA. Sea water collected from the Indian ocean in Durban. Brine shrimp eggs (*Artemia salina* Leach) were bought from a pet shop since they are readily available in these shops as food for tropical fish, and they remain viable for years in the dry state. Sodium thiopentone was purchased from Rhone-Pulenc, SA whilst Verapamil (isoptin) was purchased from Knoll AG, Germany. Isoproterenol (Isoprenaline) was purchased from Winthrop laboratory, New York whilst propranolol was bought from I.C.I Macclesfield, Great Britain. Quinidine chloride and Amiodarone hydrochloride were bought from Sigma, SA. Authentic oleanolic acid (95 % purity) and ursolic acid (97 % purity) were purchased from Africa International Food and Cosmetic Technologies (AfrIFaCT).

## 3.3 PLANT IDENTIFICATION AND COLLECTION OF PLANT MATERIAL

Fresh leaves of 16 different plant species were collected in different areas in Kwazulu-Natal province between March and July 2001 (see Table 2). The plants were identified and authentificated by a botanist, Prof. H. Baijnath, before been deposited as herbarium specimens and assigned collector's number no. MHC1-16 in the Ward Herbarium at the University of Durban-Westville. The 16 plants are; *Psidium guineense, Psidium guajava*

(white fruit), *Psidium durbanse* (hybrid), *Psidium guajava* (pink fruit), *Plantago major, Psychotria serpeus, Plantago lanceolata, Clerodendrum trichotomum., Eugenia jambulana, Buddleja salviifolia., Combretum molle, Centella asiatica, Tetradenia riparia, Syzygium cordatum, Prunus africana and Terminalia sericea.*

**Table2**.Dates, location, mass and voucher number of plant materials collected

| Name of Plant | Date Collected | Location | Weight of Crushed Leaves (g) | Herbarium Specimen Voucher Number |
|---|---|---|---|---|
| *Psidium guajava* (small fruit) | 14-03-01 | Opposite nursery at dog section of UDW | 350.0 | MH/01 |
| *Psidium guajava* (white fruit) | 14-03-01 | Road to bio-kinetic building, UDW | 303.39 | MH/02 |
| *Psidium guajava* (Hybrid) | 14-03-01 | Opposite T-junction to sports centre | 350.0 | MH/04 |
| *Psidium guajava* (pink fruit) | 16-04-01 | Near junction of Essex terrace & road to UDW | 755.0 | MH/03 |
| *Plantago major* | 17-04-01 | Mboweni | 635.0 | MH/08 |
| | 10-06-01 | Paddock sugar | | |
| | 10-06-01 | Packlane | | |
| *Psychotreia serpeus* | 03-06-01 | Isipingo | 900.0 | MH/16 |
| *Plantago lanceolata* | 16-04-01 | Along freeway & Chatsworth | 1431.0 | MH/09 |
| *Clerodendreum trichotomum* | 10-06-01 | 25km from Port Shepstone | 1383.0 | MH/07 |
| *Eugenia jambulana* | 17-04-01 | Entrance to Silvergrane road | 1381.0 | MH/06 |
| *Buddleja s.* | 20-04-01 | 200m from UDW rector's house | 364.0 | MH/13 |
| *Combretum molle* | 20-04-01 | 300m from UDW rector's house | 301.0 | MH/12 |
| *Centella asiatica* | 18-04-01 | Nursery grounds at UDW dog section | 384.0 | MH/14 |
| *Tetradenia riparia* | 17-04-01 | Silvergrane nature reserve | 142.0 | MH/15 |
| *Syzygum cordatum* | 21-04-01 | Behind botany department, UDW | 1648.0 | MH/05 |
| *Prunus africana* | 14-06-01 | Silvergrane nature reserve | 964.0 | MH/11 |
| *Terminalia sericea* | 10-06-01 | Japanese gardens, Durban | 663.0 | MH/10 |

## 3.4 BIOASSAY-DIRECTED ISOLATION OF ACTIVE PRINCIPLES OF PLANTS

### 3.4.1 Extraction of plants

The leaves from all the collected plants were air-dried, and milled into fine powder using a commercial blender. The powder of each plant was allowed to stand sequentially in hexane, dichloromethane (DCM) ethyl acetate and methanol. This exhaustive extraction was done under room temperature. The powder stood in the solvent for two days before filtering, using a 30cm filter paper (Whatman, England). The filtrate was then concentrated *in vacuo* using a rotary evaporator at a temperature of 55 $^{0}$C. The concentrated extract was recovered using minimal amounts of chloroform before being air-dried. The extraction was repeated 3 times and the extracts were combined to form four crude extracts per plant (refer Figure 14)

### 3.4.2 Thin layer chromatography

*

The crude plant extracts were analysed by TLC to reveal the type of chemical constituents they contain. This technique was carried out by spotting a diluted solution of each crude extract on a TLC plate with authentic oleanolic acid, ursolic acid, uvaol, and methyl maslinate. The TLC plate was developed with ethyl acetate/hexane (8:2) in a TLC tank.

The developed TLC plate was visualised by initial exposure to ultraviolet light before spraying with anisaldehyde/sulphuric acid/alcohol solution* and heated at 110 °C for 5 minutes. Appearance of a blue or violet-blue colouration indicated the presence of triterpenoids (Hostettmann and Marston, 1995).

* This spray reagent was prepared prior to use by mixing equal volumes of a 9:1 solution of ethanol/anisaldehyde and 9:1 ethanol/concentrated Sulphuric Acid

This led to the isolation of different compounds like ursolic acid and oleannolic acid. Ethyl acetate crude extracts were initially selected over the other crude extracts because preliminary screening showed strong presence of triterpenoids in this fraction (personal communication from Prof. F.O Shode).

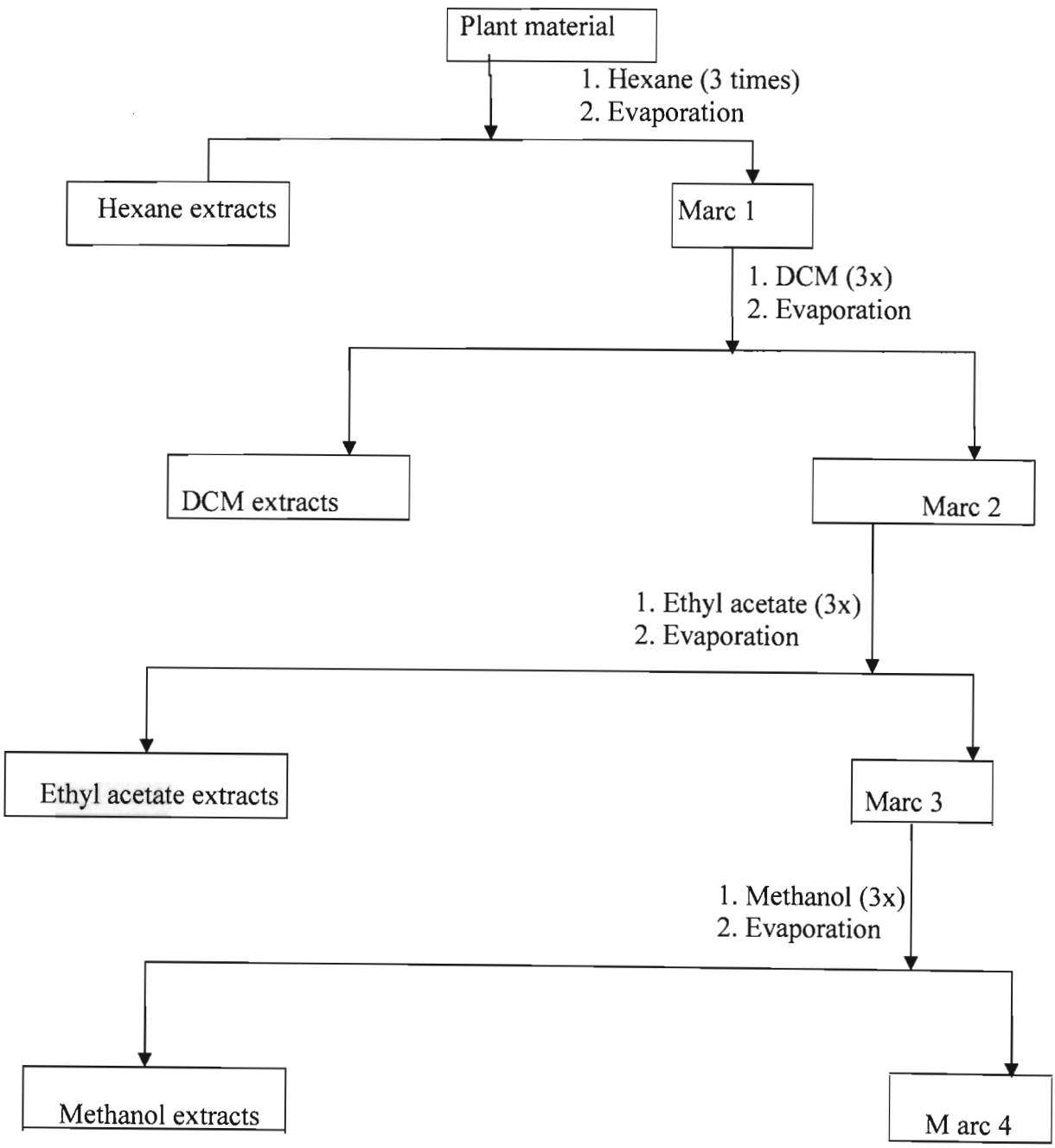


Figure 14. Schematic presentation of the sequential crude extraction using different solvents.

### 3.4.3 Isolation and purification of compounds

Since preliminary experiments had indicated that the ethyl acetate crude extracts contained most of the active compounds, that is the triterpenoids, only the ethyl acetate crude extracts was subjected to further purification process. The crude extracts were sequentially fractionated on silica gel 60 (particle size 0.0063- 0.200 mm) column chromatography with gradient elution (100 % hexane to 60 % hexane/ethyl acetate). All the collected fractions were subjected to a TLC analysis together with authentic triterpenoids. Fractions were pooled according to similar TLC profiles and the pooled fractions were concentrated *in vacuo* using a rotary evaporator at 50 $^{0}$C. The concentrates were reconstituted using minimal amounts of chloroform and allowed to air dry in preweighed vials.

### 3.4.4 STRUCTURE ELUCIDATION

The eluates that had similar TLC profiles to the authentic triterpenoids were combined and subjected to further chromatographic purifications. This process produced 80 % - 98 % pure isolates. These isolates were subjected to spectroscopic analysis using $^{1}$H and $^{13}$C NMR techniques.

The chemical structures of the isolates were revealed when their spectral data were compared with the spectral data of authentic triterpenoids namely; oleanolic acid, ursolic acid, uvaol, methyl maslinate, methyl corosolate and betulunic acid

## 3.5 TEST FOR TOXICITY

All the crude ethyl acetate extracts and the pure triterpenoids from the plants collected were subjected to brine shrimp bioassay test according to the method of Meyer *et al* (1982). $LC_{50}$'s and 95 % confidence interval was determined from the 24 hour counts of the survived naupii by intersection.

### 3.5.1 Hatching the Shrimp

Two teaspoons of brine shrimp eggs were sprinkled into the one side of a beaker containing natural sea water that was continuously being aerated with compressed air. Covering, with aluminium foil, the sides and top, darkened one side of the beaker. The eggs were allowed 48 hours to hatch.

### 3.5.2 Sample preparation

Samples were prepared by dissolving 20 mg of extracts and the pure triterpenoids in 2 ml of methanol. Appropriate amounts of solution (5 µl, 50 µl, and 500 µl for 10, 100, and 1000 µg/ml, respectively) transferred to 1.25 cm discs of filter paper. Control discs were prepared using only methanol. Three replicates were prepared for each dose level. The discs were placed in vials and then dried in an oven for 1 hour.

### 3.5.3 Bioassay

After 48 hours, 10 shrimp were transferred to each sample vial (30 shrimp per dilution) using a disposable pipette, and seawater was added to make 5 ml. The vials were

maintained at 37 $^{0}$C. Survivors were counted after 24 hours, and the percent deaths at each dose and control were determined.

### 3.6- ANIMALS USED

Wistar male rats weighing between 300 and 320g were used. These rats were housed in the University of Durban-Westville's Biomedical Resource Centre. They were exposed to the following physico-chemical factors; a 12 hour light: 12hour dark cycle, temperature of 21° C ± 2, carbon dioxide content of <5 000 p.p.m., ammonia content of <50 p.p.m., noise levels of < 60 decibels, and under constant relative humidity of 50 % ± 5. Water and standard pellet food were provided *ad libitum.* The bedding comprised of chemically neutral, dust free sawdust and wood chips, gamma irradiated at 2,5 Megarads. Before the experiments, the animals were fasted overnight.

Guinea pigs of all sexes that were above 600g were used. The guinea pigs were respernised and fasted overnight. The reserpine was administered to elucidate whether the positive inotropic was attributable to an indirect sympathomimetic action since reserpine deplete endogenous junctional noradrenaline stores (Salzmann *et al.*, 1985). They were provided with water and food *ad libitum.*

### 3.7- URINARY PARAMETERS IN RATS

The animals were placed in individual Nalgene metabolic cages in order to collect excreted urine for electrolyte analysis. The test compound was administered intraperitoneally at a dose of 60 mg/kg in 5 ml distilled water per kg body weight, and the urea was applied in a dose of 1 g/kg body weight. Urine excretion was recorded 5 and 24 hours after the

administration of the test compound. The electrolyte content of the urine was analysed using Beckman Synchron EL-ISE Electrolyte System (Germany). Six test animals were used per group.

### 3.7.1 Diuretic activity in rats (LIPSCHITZ test)

The test is based on water and sodium excretion in test animals and compared to rats treated with a high dose of urea. The "Lipschitz-value" of diuretic activity is the quotient between excretion by test animals (6 per group) and excretion by the urea control (Vogel and Vogel, 1997).

In order to evaluate the diuretic effect, urine volume excreted per 100 g body weight was calculated for each group. Results were expressed as the "Lipschitz-value", i.e., the ratio T/U, in which T is the response of the test compound, and U is that of the urea treatment. Indices of 1.0 and more was regarded as a positive effect. With potent diuretics, values of 2.0 and more can be obtained. For Saluretic drugs like hydrochlorothiazide, values around 1.8 are reported whilst loop diuretics like furosemide, values of 4.0 or more are expected. In order to indicate the duration of the diuretic effect, the "Lipschitz-value" were calculated for both the 5 and 24 hour excretion period.

### 3.7.2 Saluretic parameters

Excretion of electrolytes is as important as the excretion of water for the treatment of hypertension. Saluretic drugs and potassium-sparing diuretics were developed to prevent potassium loss. The diuresis test in rats was modified in such a way that potassium and chloride as well as osmolality are determined in addition to water and sodium (Vogel and

Vogel, 1997). Ratios between electrolytes can be calculated indicating carbonic anhydrase inhibition or a potassium sparing effect.

Just as in the Lipschitz test, the 5 and 24 hour urine was collected and analysed using Beckman Synchron EL-ISE Electrolyte System (Germany). Hydrochlorothiazide (25 mg/kg body weight) was used as a standard. From the electrolyte content in the urine, the sum of $Na^+$ and $Cl^-$ excretion was calculated as a parameter for saluretic activity. The ratio $Na^+/ Cl^-$ was calculated for natriuretic activity. Values greater than 2.0 indicated a favourable Natriuretic effect whilst ratios greater than 10.0 indicated a potassium-sparing effect. The ratio $Cl^-/ Na^+ + K^+$ (ion quotient) was calculated to estimate carbonic anhydrase effect. Carbonic anhydrase inhibition was excluded at ratios between 1.0 and 0.8 whilst with decreasing ratios slight to strong carbonic anhydrase inhibition was assumed.

## 3.8 BLOOD PRESSURE MEASUREMENTS

Systolic, diastolic and mean blood pressures in each conscious wistar rat were determined by indirect method using tail-cuff method and by the direct method.

### 3.8.1 Indirect BP measurements

Four wistar rats were used per test compound, in addition to six untreated control wistar rats. Before recording the actual experimental readings, the rats were trained for four consecutive days to minimise stress reactions and thus increasing the reliability of our results. The training regime comprised the whole procedure that would be followed during

the actual readings. A tail-cuff computerised blood pressure monitor (IITC Life Sciences 31, USA) was used.

The blood pressure monitor works with an IITC hardware system to determine both the blood pressure and the heart rate. Typically the system employs a an automatic scanner and pump in addition to a photoelectric sensor in the tail sensing cuff and amplifier to measure and count the heart beat pulses in the animals tail. The use of the photoelectric sensor permits measurement at the lowest ambient temperature possible thereby reducing heat stress on the animal and as such yielding results that closer to the values obtained by cannulation. The results were displayed as data plots and summary data of systolic, diastolic and mean blood pressures, and heart rate on the computer screen. We used the medium restraining devises and the 15 mm tail cuffs in accordance with the rats' size. Three closes readings were averaged for each rat per session, and the measurements were done at the same time of day to prevent circadian rhythmic changes in pressure. The room temperature was kept 30 °C and the restraining devices were warmed before placing the animal. This method was validated in our laboratory by simultaneous direct (cannulation through the left common carotid artery) and indirect (tail-cuff method) measurements of blood pressure as is required according to Buñag (1991). There was good correlation between direct and indirect blood pressure measurement. For systolic blood pressure $r = 0.96$, for diastolic blood pressure $r = 0.67$, and for heart rate $r = 0.87$.

Two types of measurements were performed:

- After a single intraperitoneal application of the test compound , systolic and diastolic blood pressure and heart rate were monitored for 60 minutes in 10

minutes intervals so as to record the effects of the compound and also the duration of the effect.

- The same parameters were monitored for 6 consecutive days just before the animals received a daily single intraperitoneal (i.p.) injection of the test compound..

### 3.8.2 Direct BP measurements

Six anaesthetised wistar rats were used to detect the effect of the test compounds on blood pressure and heart rate. The rats were anaesthetised by intraperitoneal (i.p.) injection of sodium thiopentone, 40 mg/kg body weight (Rhone-Pulenc, SA). For continuous monitoring of mean arterial blood pressure and heart rate, the left carotid artery was cannulated and a catheter was connected to a pressure transducer (Statham MLT 0380, Ad instruments), compatible with the PowerLab System ML410/W. The electrocardiogram (ECG) was monitored throughout the experiments (ECG-C1, ESAOTE Biomedica, Italy, and PowerLab System ML410/W. Changes in ECG were recorded from standard lead II only at 25-mm/sec chart velocity. The evaluation of inotropic and dromotropic effects, and action potential duration was based on the computer analysis of the ECG registered by the PowerLab System. Blood pressure values were expressed as means of three consecutive measurements

## 3.9 BIOCHEMICAL DETERMINATIONS

Blood glucose in whole blood was estimated by Glucometer Elite, Bayer Diagnostics, France. The blood was collected from the cut tail tip 30 minutes after intraperitoneal administration of the test compound.

## 3.10 CARDIOTONIC EXPERIMENTS

In all experiments, authentic OA (95 % purity) and UA (97 % purity) were used a reference substances for the tested triterpenoids

### 3.10.1 Cardiotonic measurements in guinea pig atria

Guinea pigs of either sex weighing 400-600 g were used to study the cardiotonic effects of the triterpenoids and the plant extracts according to the method described by Gilani *et al.* (1999). The guinea pigs were reserpinised (1 mg/kg) 24 h before being sacrificed by cervical dislocation. The reserpine was administered to elucidate whether the positive inotropic was attributable to an indirect sympathomimetic action since reserpine deplete endogenous junctional noradrenaline stores (Salzmann *et al.*, 1985). The heart was quickly removed and placed in oxygenated physiological solution (Krebs-Hanseleit buffer). The left and right atria were separated carefully and cleaned of fatty tissue. The preparation was fixed to a bipolar platinum point electrode mounted in a 20 ml tissue chamber containing the physiological salt solution at 37°C, and was constantly being aerated by carbogen ($O_2$ containing 5% $CO_2$). Right atria were allowed to beat spontaneously under a resting tension of 1 g, and the left atria were electrically stimulated by 0.7 ms pulses of 10-15 V, 2 Hz. All the preparations were equilibrated for a 30 minute period before the addition of any test compound. Isometric contractions and, in right atria, rates of spontaneous beating were recorded by means of a force-displacement transducer (Ugo Basile, Italy) on a recorder (Ugo Basile, Italy)

For the evaluation of cardiotonic responses, the following protocol was used: After the 30 minutes equilibration period, the atrium was thoroughly washed (at least 3 times) and its

viability was tested by administering isoprenaline (0.1, 0.25 µl) as a reference substance. The tissue was then washed 3 times and equilibrated for 15 minutes and it was washed a further 3 times. After baseline values were obtained, cumulative doses of each test compound were added to the tissue bath, and its inotropic and chronotropic effects were recorded for a total of 10 minutes. The test compounds were added to the tissue bath in a cumulative fashion (0.05, 0.50, 1.00 mg/ml) at 3 minute interval to obtain a complete dose-response curve. The tissue was then washed three times and equilibrated for a 15 minutes before being checked for its viability and comparison with effect of isoprenaline. Upon being washed thoroughly, the tissue was further equilibrated for a 15 minutes and washed again before testing another compound. Each tissue was used for a maximum of four test compounds.

### 3.10.2 β-adrenergic effects of crude plant extracts and pure compounds

To see if the inotropic effects of the extracts and the pure triterpenoids were mediated through a β-adrenergic -like mechanism, the atria was pre-treated with propranolol (1 µM), a β-adrenoceptor blocking agent (Hoffman and Lefkowitz, 1990). The test compounds were applied 5 minutes after the application of propranolol to allow complete block of the β-receptors.

We also checked whether the test compounds could either block or potentiate the effect of isoprenaline. This was done by firstly obtaining baseline values of the tissue then cumulative dose of isoprenaline (0.1, 0.25 and 0.5 µl) followed by the test compounds. 5 minutes later, isoprenaline administration was repeated and compared to the control values.

### 3.10.3 Cholinergic experiments

To study the involvement of cholinergic receptors in the mechanism of action of our test compounds on the guinea pig myocardium, 5µg/ml of atropine sulphate was used. Involvement of the cholinergic mechanism was performed by introducing the extracts before and after adding the atropine sulphate to the tissue bath.

## 3.11- ANTIDYSRHYTHMIC EXPERIMENTS

The effects were evaluated on chemical and ischaemia-reperfusion models of arrhythmia. For chemically induced arrhythmia, we used the $CaCl_2$-, $BaCl_2$-, and adrenaline induced models of arrhythmia. For the ischaemia-reperfusion induced arrhythmia, we used the coronary artery ligation/reperfusion model. The Lambeth convention's guidelines were followed (Walker *et al.*, 1988). As positive controls, we used all four classes of antiarrhythmic reference drugs according to the Vaughan Williams classification (1991). The reference drugs used were: for class I (sodium fast channel blockers) quinidine chloride was used; for class II (β-adrenergic antagonists), propranolol hydrochloride was used; for class III (prolonged action potential by blocking potassium channels) amiodarone hydrochloride was used; for class IV (slow calcium channel blocker) verapamil was used. The optimal dosages for the animals are shown together with the results and were determined during the preliminary experiments. These drugs were administered through the cannulated right jugular vein. The drug administration was standardised to injections of 0.2 ml per 100 g body weight over a period of 1 min to prevent inducing arrhythmias.

In the intact animal, all the information necessary for the detection of arrhythmias is adequately provided by a single ECG lead with sufficient resolution (Walker *et al.*, 1988).

Thus, in accordance with the Lambeth conventions, the ECG (ECG-C1, ESAOT Biomedica, Italy) itself was taken as a sole basis for definition, classification, and quantification of arrhythmia. Changes in ECG were registered with needle electrodes only from the standard lead II at 25-mm/sec chart velocity. The ECG records were kept in the department where they will be for at least the next seven years as is postulated by the Lambeth Conventions.

### 3.11.1 Chemically induced models of arrhythmia

To test the anti-arrhythmic effect of our test compounds on the chemically induced arrhythmia, six anaesthetised rats were used per compound. The rats were anaesthetised by intraperitoneal injection of sodium thiopentone (40 mg/kg body weight). All the chemicals used to induce the arrhythmias were administered through the cannulated right jugular vein. The arrhythmias were induced 20 minutes after intraperitoneal administration of a test compound. This was done like this to check whether the test compounds had any prophylactic potential on the occurrence of the chemically induced-arrhythmias. Before the application of the test compound, control ECG of each arrhythmogenic chemical was registered.

#### 3.11.1.1 $BaCl_2$ –induced arrhythmia

$BaCl_2$-induced arrhythmias were used to investigate the antiarrhythmic action of our test compound on the rhythmicity of the myocardium and prolongation of the action potential by decreasing the efflux of potassium. Unlike the dosage of 2 mg/kg as was used by

Petkov and Manolov (1972), a dosage of 1 mg/kg was used in this study because it was found in preliminary experiments that the former dose killed the rats. $BaCl_2$ (1 mg/kg) was administered intravenously 20 minutes after intraperitoneal administration of the test compounds. Control ECG of $BaCl_2$ alone was recorded so as to compare it with the ECG obtained from the $BaCl_2$ applied on the background of the test compound.

#### 3.11.1.2 $CaCl_2$ – induced arrhythmia

$CaCl_2$-induced arrhythmia was used to evaluate possible antiarrhythmic calcium antagonistic effects of the test compounds. The arrhythmia was initiated by administering a 2.5 % $CaCl_2$ solution (140 mg/kg) (Papp *et al.*, 1966) on the background of the test compound that was administered 20 minutes before. Changes in ECG were recorded after the application of $CaCl_2$ before and on the background of the test compounds

#### 3.11.1.3 Adrenaline-induced arrhythmia

Adrenaline-arrhythmia in rats were induced through intravenous injection of adrenaline in a dose of 10 μg/kg for the evaluation of β-blocking activity (Barrett and Cullum, 1968). Just like above, changes in ECG were recorded after the application of $CaCl_2$ before and on the background of the test compounds.

### 3.11.2- Ischemia-reperfusion arrhythmia

To evaluate the effect of the test compounds on the ischaemia-reperfusion model of arrhythmia, six anaesthetised rats were used per each compound. The rats were

anaesthetised by intraperitoneal injection of sodium thiopentone (40 mg/kg body weight). The trachea was cannulated and artificial respiration was achieved by a positive-pressure rodent respirator (Phipps and Bird Inc., USA). This artificial respiration used atmospheric air at a tidal volume of approximately 5 ml at a rate of 25 breaths per minute. Throughout the whole experiment, changes in ECG (ECG-C1, ESAOT Biomedica, Italy) were monitored from standard limb lead II with needle electrodes. Haemodynamic parameters (mean arterial pressure and heart rate) were monitored from a cannulated carotid artery with a catheter connected to a Statham MLT 0380 pressure transducer (Ad instruments), compatible with the PowerLab System ML410/W, Australia.

Regional myocardial ischaemia model of arrhythmia and the reperfusion model of arrhythmia were used since as has been stated in chapter two, these two arrhythmias are initiated by different mechanisms (Curtis and Hearse, 1989). Ischaemia-reperfusion arrhythmias were induced according to the method described by Kane *et al.*, (1984) with slight modifications. In anaesthetised and artificially ventilated rats, the chest was opened using a left thoracotomy, followed by sectioning of ribs 4 and 5, approximately 2 mm to the left of the sternum. Positive pressure artificial respiration was started immediately to maintain normal $PCO_2$, $PO_2$ and pH parameters. After incising the pericardium, the heart was exposed out of the chest using gentle pressure on the rib cage. A 6/0 silk non-traumatic suture was passed through the epicardial layer under the left main coronary artery, about 2 mm from the origin. A small plastic button was threaded through the ligature and placed in contact with the heart. The end of the ligature was passed through a small vinyl tube and exteriorised. The heart was massaged before being replaced in the chest, and then thorax was closed after removing the residual air to avoid pneumothorax. The animal was left to recover for 15 minutes. Any animal in which this procedure

produced arrhythmias or a sustained fall in blood pressure to less than 70 mmHg was discarded. Applying tension to the ligature could then occlude the artery and reperfusion was achieved by releasing the tension

Two types of studies were undertaken for the antiarrhythmic actions of our test compounds. In the first type of experiments, the artery was occluded for 30 minutes to induce experimental ischaemia, and then the tension was released for 20 minutes to produce reperfusion. In this study, any heart that was not in sinus rhythm during 2 seconds just before and at the moment of reperfusion was excluded from the reperfusion study and was immediately replaced (Walker *et al.*, 1988; Curtis and Hearse, 1989). The ischaemia were done either 20 minutes after intraperitoneal administration of the test compound or 10 minutes after intravenous injection of the antiarrhythmic drugs. In the second set of experiments, the drug pre-treatment was replaced by ischaemic preconditioning produced by three 3-minutes occlusion periods interspaced with 3-mnutes reperfusion period. After the last 3-mute reperfusion, the artery was occluded for 30 minutes (to produce ischaemia) followed by 20 minutes reperfusion.

To determine whether reperfusion was in fact occurring, 0.1 ml $100^{-1}$ (body weight) of a 10 % solution of fluorescein (Sigma) was administered through the jugular vein immediately before sacrificing the animals. The heart was rapidly removed from the chest and placed in ice cold KCl solution (10 % weight/volume) for 1 min to cause cardioplegia. A gross examination of the heart was made under ultraviolet light to determine whether areas of non-perfused myocardium were present. These appeared dark blue under ultraviolet light whereas perfused were stained green. The hearts were then photographed under ultraviolet light to give a permanent record. As a comparison, this procedure was also carried out in

hearts with a permanent ligation, after 30 minutes of ischaemia, and in control non-ischaemic hearts.

### 3.12- STATISTICAL ANALYSIS

All results were evaluated originally as means ± SEM. After statistical analysis, the results were presented, when indicated, as % of the baseline value. For all analyses, INSTAT V2.04 program was used, including one-way and two-way ANOVA, *t*-test and Chi-square test. A p-value less than 0.05 was considered to be statistically significant.

# 4.0 RESULTS

## 4.1 PLANT MATERIALS AND EXTRACTS

The extraction process yielded four different crude extracts per plant. The four types of extracts were; the hexane crude extracts, chloroform crude extracts, ethyl acetate crude extracts and the methanol crude extracts. Thus, 64 different crude extracts from 16 different medicinal plants were obtained. The weight of these crude extracts are shown in appendix 2. As was indicated in the methodology section in chapter 2, the preliminary studies had shown that the crude ethyl acetate extracts contained many of the triterpenoids than the other extracts. Thus, we only used the ethyl acetate crude extract to investigate the cardiovascular effects of them. We also used the ethyl acetate crude extracts to isolate and purify the triterpenoids.

Both TLC and NMR (appendix 5) revealed that indeed the crude ethyl acetate extracts contained a number of triterpenoids. Table 3 shows the approximate percentage of the triterpenoids extracted from the crude ethyl acetate extract. All the plants contained triterpenoids though in different concentration. The highest yield was obtained from *Buddleja salviifolia* followed by *Syzygum cordatum* then *Psidium guajava* (white fruit) and *Eugenia jambulana*. The least amount of triterpenoids was obtained from *Centella asiatica* followed by *Tetradenia riparia* then *Terminalia sericea* and *Clerodendrum*. The isolation and purification processes yielded the following triterpenoids; oleanolic acid, ursolic acid, uvaol and methyl maslinate (Figure 15). Oleanolic acid was the most predominant of all the triterpenoids. Oleanolic acid (OA) recrystallised from methanol as colourless crystals with a melting point of about 307-308 $^{o}$C. Its spectral data (Fig. 42) were identical with

literature values (Maillard *et al.*, 1992). Ursolic acid (UA) recrystallised from methanol as colourless crystals with melting point of 286 $^{0}$C. Its spectral data (Fig. 43) were identical with literature values (Seo *et al.*, 1975a). Uvaol (UV) recrystallised from methanol/dichloromethane as colourless crystals with a melting point of about 221-223 $^{o}$C. Its spectral data (Fig. 44) were identical with literature values (Siddiqui *et al.*, 1986). Methyl maslinate (MM) was only eluted when the crude ethyl acetate extract was subjected to a silica gel column chromatography with gradient elution using a 60 %/ 40 % hexane/ethyl acetate solvent system. The eluates containing MM were obtained only from the last fractions. MM was used without recrystallisation, and its spectral properties (Fig. 45) were identical with literature values (Seo *et al.*, 1975b)

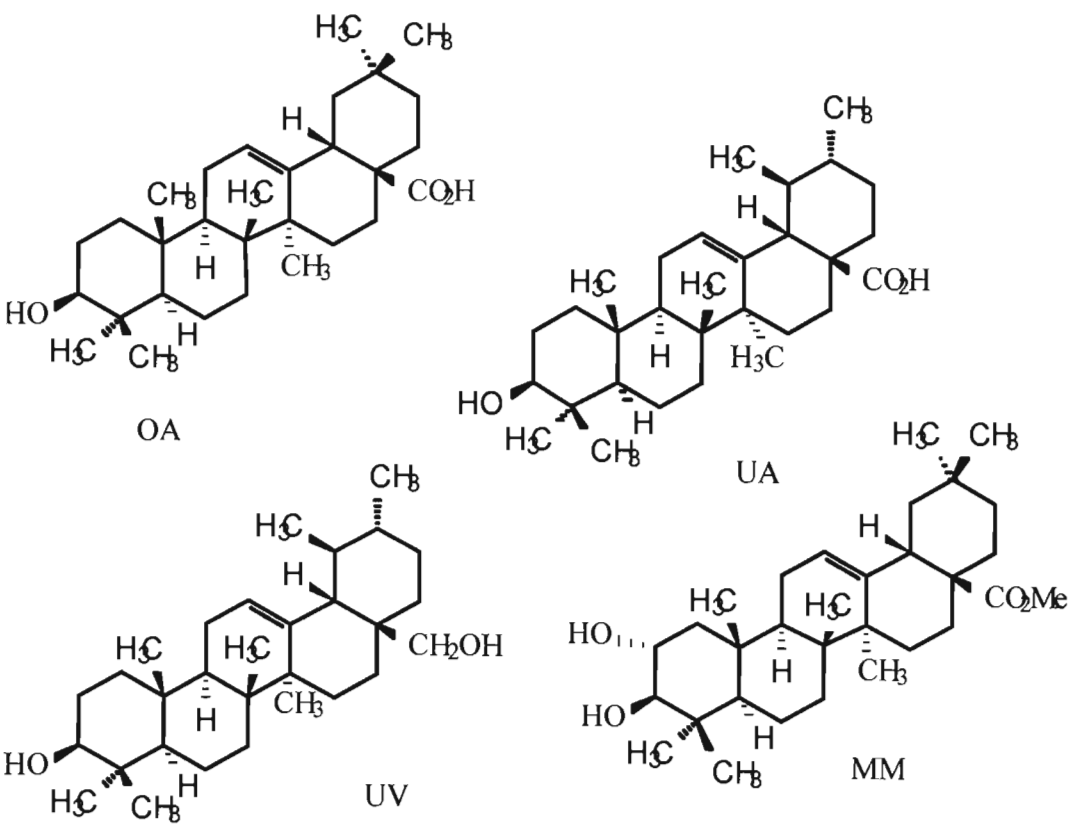


Figure 15. Structures of oleanolic acid (OA), ursolic acid (UA), uvaol (UV) and methyl maslinate (MM)

**Table 3: Approximate percent yield of triterpenoids from crude ethyl acetate extracts from different plants**

| NAME OF PLANT | ESTIMATED % YIELD OF TRITERPENOIDS |
|---|---|
| *Psidium guajava* (small fruit) | 0.29 |
| *Psidium guajava* (white fruit) | 0.37 |
| *Psidium guajava* (pink fruit) | 0.13 |
| *Psidium guajava* (hybrid) | 0.15 |
| *Syzygum cordatum* | 0.62 |
| *Eugenia jambulana* | 0.33 |
| *Clerodendrum L* | 0.04 |
| *Plantago major* | 0.25 |
| *Plantago lanceolata* | 0.11 |
| *Terminalia sericea* | 0.04 |
| *Prunus africana* | 0.29 |
| *Combretum molle* | 0.05 |
| *Buddleja salvifolia* | 0.69 |
| *Centella asiatica* | 0.01 |
| *Tetradenia riparia* | 0.02 |
| *Psychotreia serpeus* | 0.09 |

## 4.2. TOXICITY RESULTS

Acute toxicity of the ethyl acetate crude extracts from all the 16 plants and three triterpenoids: oleanolic acid, ursolic acid and uvaol was done using brine shrimp (*Artemia salina)* bioassay. We were unable to do toxicity test of methyl maslinate (MM) because it was not dissolving in methanol, and it could only dissolve in DMSO. However, DMSO proved to be toxic on its own and hence could not be used as a solvent for testing the toxicity in brine shrimp. On the other hand, all the crude ethyl acetate extracts from the 16 plants, and OA, UA and UV showed very low toxicity. The $LC_{50}$ of them are shown in table 4 below. On the basis of the low toxicity of all the tested substances, we assumed that MM had also low toxicity, and hence it was applied in the experiments at the same doses as the other compounds.

**Table 4: $LC_{50}$ of phytochemical extractives and authentic oleanolic acid, ursolic acid, uvaol and methyl maslinate evaluated on brine shrimp test**

| Group | $LC_{50}$ (mg/ml) |
|---|---|
| Oleanolic acid | 0.100 ± 0.035 |
| Ursolic acid | 0.950 ± 0.040 |
| Uvaol | 1.100 ± 0.040 |
| Methyl maslinate | - |
| *Psidium guajava* (small fruit) | 0.850 ± 0.035 |
| *Psidium guajava* (white fruit) | 1.500 ± 0.045 |
| *Psidium guajava* (pink fruit) | 1.000 ± 0.050 |
| *Psidium guajava* (hybrid) | 0.600 ± 0.045 |
| *Syzygum cordatum* | 1.200 ± 0.050 |
| *Eugenia jambulana* | 0.100 ± 0.045 |
| *Clerodendrum capense* | 0.100 ± 0.045 |
| *Plantago major* | 0.100 ± 0.046 |
| *Plantago lanceolata* | 0.700 ± 0.035 |
| *Terminalia sericea* | 6.000 ± 0.080 |
| *Prunus africana* | 3.300 ± 0.085 |
| *Combretum molle* | 5.200 ± 0.080 |
| *Buddleja salvifolia* | 1.800 ± 0.040 |
| *Centella asiatica* | 1.800 ± 0.040 |
| *Tetradenia riparia* | 4.000 ± 0.065 |
| *Psychotria serpens* | 0.010 ±0.004 |

**$LC_{50}$- Mean ± SEM**

## 4.3. EFFECTS ON URINARY PARAMETERS

The urinary analysis for the diuretic, saluretic and natriuretic effects of oleanolic acid, ursolic acid and the phytochemicals are presented in table 5a and 5b. Table 5a are the

results from the urine that was collected 5 hours after intraperitoneal administration of the test compounds whilst table 5b contains analysis of the urine that was collected 24 hours after intraperitoneal injection of the test compounds. No carbonic anhydrase inhibition was detected.

### *4.3.1* Diuretic effects of the test compounds

The results of the Lipschitz test analysed using the urine collected 5 hours after intraperitoneal injection indicate that all the test compounds except *Psidium guajava* (small fruit) and *Psidium guajava* (white fruit) had a diuretic effect that was comparable to that of hydrochlorothiazide (Figure 5a). On the other hand, the results from the urine collected 24 hours after intraperitoneal administration show that only, oleanolic acid, ursolic acid, *Syzygum cordatum, Eugenia jambolona, Plantago lanceolata, Tetradenia riparia* and *Psychotreia serpens* exhibited a diuretic effect that was comparable to that of hydrochlorothiazide (Figure 5b).

### *4.3.2* Saluretic effects of the test compounds

Table 5a show the saluretic effects of the test compounds after 5 hours intraperitoneal application of them. It can be seen from these results that only ursolic acid displayed a slight saluretic effect on the animals. Analysis of the urine collected after 24 hours indicate that only Eugenia jambolona and Psidium guajava (small fruit) had a saluretic effect that was comparable to that of hydrochlorothiazide (Table 5b).

**TABLE 5a: Diuretic, Saluretic and Natriuretic activity of oleanolic, ursolic and phytochemical extractives (5 hours after intraperitoneal administration)**

| Group/Parameter | Diuresis (ml/100g b.w) | Lipschitz Value (T/U) | Na + Cl (mmol/l) | Na/K (mmol/l) | $\frac{Cl}{Na + K}$ (mmol/l) |
|---|---|---|---|---|---|
| Urea (1g/kg b.w.) | 1.4 ± 0.2 | - | 440 ± 12.6 | 1.67 ± 0.17 | 0.654 ± 0.02 |
| Hydrochlorothiazide (25mg/kg b.w) | 3.5 ± 0.6 | 2.50 | 520 ± 8.4 | 3.44 ± 0.10 | 0.834 ± 0.02 |
| Oleanolic acid (60 mg/kg) | 1.4 ± 0.2+ | 1.00 | 376 ± 15.7*+ | 1.07 ± 0.12+ | 0.618 ± 0.05+ |
| Ursolic acid (60 mg/kg) | 1.6 ± 0.3+ | 1.10 | 442 ± 12.8 + | 1.47 ± 0.17+ | 0.466 ± 0.03*+ |
| *Psidium guajava* (small) | 0.80 ± 0.10*+ | 0.58 | 297 ± 12.0*+ | 0.90 ± 0.11*+ | 0.525 ± 0.06*+ |
| *Psidium guajava* (white) | 1.10 ± 0.2+ | 0.79 | 353 ± 19.0*+ | 0.86 ± 0.05*+ | 0.610 ± 0.01*+ |
| *Psidium guajava* (pink) | 2.60 ± 0.2 | 1.86 | 241 ± 7.4*+ | 1.24 ± 0.08+ | 0.544 ± 0.01*+ |
| *Psidium guajava* (hybrid) | 1.42 ± 0.10+ | 1.01 | 188 ± 12.5*+ | 0.92 ± 0.10*+ | 0.572 ± 0.01*+ |
| *Syzygum cordatum* | 1.67 ± 0.2+ | 1.19 | 246 ± 406*+ | 1.32 ± 0.13+ | 0.530 ± 0.02*+ |
| *Eugenia jambulana* | 1.86 ± 0.2+ | 1.33 | 242 ± 13.1*+ | 1.26 ±0.30+ | 0.480 ± 0.01*+ |
| *Clerodendrum L* | 2.61 ± 0.2 | 1.86 | 232 ± 7.5*+ | 1.49 ± 0.06+ | 0.526 ± 0.01*+ |
| *Plantago major* | 2.11 ± 0.3 | 1.51 | 225 ± 1.0*+ | 1.26 ± 0.14+ | 0.522 ± 0.01*+ |
| *Plantago lanceolata* | 2.28 ± 0.3 | 1.63 | 227 ± 12.0*+ | 1.55 ± 0.50+ | 0.548 ± 0.05*+ |
| *Terminalia sericea* | 2.67 ± 0.2 | 1.91 | 233 ± 12.6*+ | 1.29 ± 0.25+ | 0.502 ± 0.01*+ |
| *Prunus africana* | 3.19 ± 0.3 | 2.28 | 2.71 ± 8.2*+ | 1.44 ± 0.08+ | 0.508 ± 0.01*+ |
| *Combretum molle* | 2.74 ± 0.2 | 1.96 | 239 ± 7.6*+ | 1.38 ± 0.01+ | 0.510 ± 0.02*+ |
| *Buddleja salvifolia* | 2.15 ± 0.2 | 1.54 | 282 ± 2.6*+ | 1.10 ± 0.11+ | 0.569 ± 0.01*+ |
| *Centella asiatica* | 2.52 ± 0.3 | 1.80 | 249 ± 10.0*+ | 1.50 ± 0.04+ | 0.515 ± 0.01*+ |
| *Tetradenia riparia* | 2.26 ± 0.3 | 1.61 | 256 ± 1.0*+ | 1.59 ± 0.09+ | 0.506 ± 0.01*+ |
| *Psychotreia serpeus* | 1.62 ± 0.1+ | 1.16 | 330 ± 10.0*+ | 1.12 ± 0.8+ | 0.552 ± 0.01*+ |

**Mean ± SEM. All phytochemicals were applied in a dose 100mg/kg b.w. except Psychotreia serpeus (60 mg/kg b.w.)**

*** The difference is significant compared to effect of urea**

**+ The difference is significant compared to effect of hydrochlorothiazide**

**T/U "Lipschitz-value" ratio in which T is the response of the tested compound and U is the urea response**

**TABLE 5b: Diuretic, Saluretic and Natriuretic activity of oleanolic, ursolic and phytochemical extractives (24 hours after intraperitoneal administration)**

| Group/Parameter | Diuresis (ml/100g b.w) | Lipschitz Value (T/U) | Na + Cl (mmol/l) | Na/K (mmol/l) | $\frac{Cl}{Na + K}$ (mmol/l) |
|---|---|---|---|---|---|
| Urea (1g/kg b.w.) | 7.0 ± 0.6 | - | 281 ± 10.0 | 1.32 ± 0.12 | 0.648 ± 0.01 |
| Hydrochlorothiazide (25mg/kg b.w) | 14.6 ± 0.5 | 2.08 | 382 ± 7.6 | 2.43 ± 0.12 | 0.672 ± 0.01 |
| Oleanolic acid (60 mg/kg) | 7.0 ± 0.4+ | 1.00 | 269 ± 8.4+ | 0.92 ± 0.13+ | 0.539 ± 0.01+ |
| Ursolic acid (60 mg/kg) | 6.8 ± 0.4+ | 0.97 | 227 ± 12.2+ | 0.83 ± 0.10+ | 0.566 ± 0.02+ |
| *Psidium guajava* (small) | 2.30 ± 0.2*+ | 0.33 | 350 ± 11.0+ | 1.04 ± 0.10+ | 0.528 ± 0.02+ |
| *Psidium guajava* (white) | 2.62 ± 0.15*+ | 0.38 | 229 ± 9.0+ | 1.11 ± 0.15+ | 0.435 ± 0.02*+ |
| *Psidium guajava* (pink) | 4.86 ± 0.3*+ | 0.69 | 240 ± 1.0+ | 1.71 ± 0.16+ | 0.420 ± 0.01*+ |
| *Psidium guajava* (hybrid) | 3.03 ± 0.2*+ | 0.43 | 223 ± 10*+ | 1.41 ± 0.50+ | 0.429 ± 0.01*+ |
| *Syzygum cordatum* | 6.62 ± 0.3+ | 0.96 | 278 ± 10.0+ | 2.08 ± 0.34 | 0.428 ± 0.03*+ |
| *Eugenia jambulana* | 6.25 ± 0.2+ | 0.90 | 309 ± 11.0+ | 3.05 ± 0.16 | 0.410 ± 0.01*+ |
| *Clerodendrum L* | 3.99 ± 0.3*+ | 0.57 | 275 ± 5.9+ | 1.84 ± 0.10+ | 0.406 ± 0.01*+ |
| *Plantago major* | 4.04 ± 0.2*+ | 0.58 | 226 ± 1.7*+ | 1.96 ± 0.14+ | 0.422 ± 0.02*+ |
| *Plantago lanceolata* | 6.28 ± 0.3+ | 0.90 | 241 ± 8.9+ | 2.23 ± 0.12 | 0.410 ± 0.01*+ |
| *Terminalia sericea* | 4.25 ± 0.2*+ | 0.61 | 233 ± 10.0+ | 1.56 ± 0.14+ | 0.477 ± 0.05*+ |
| *Prunus africana* | 4.65 ± 0.1*+ | 0.66 | 211 ± 12.0*+ | 1.31 ± 0.12+ | 0.499 ± 0.04*+ |
| *Combretum molle* | 4.96 ± 0.2*+ | 0.71 | 174 ± 2.9*+ | 1.28 ± 0.06+ | 0.501 ± 0.01*+ |
| *Buddleja salvifolia* | 4.90 ± 0.3*+ | 0.70 | 239 ± 12.6+ | 1.87 ± 0.05+ | 0.443 ± 0.01*+ |
| *Centella asiatica* | 4.34 ± 0.2*+ | 0.62 | 236 ± 1.0+ | 1.66 ± 0.03+ | 0.461 ± 0.02*+ |
| *Tetradenia riparia* | 6.58 ± 0.2+ | 0.93 | 235 ± 1.4+ | 2.00 ± 0.04+ | 0.416 ± 0.04*+ |
| *Psychotreia serpeus* | 6.22 ± 0.2+ | 0.90 | 261 ± 10.0+ | 2.10 ± 0.10 | 0.411 ± 0.01*+ |

**Mean ± SEM. All phytochemicals were applied in a dose 100mg/kg b.w. except Psychotreia serpeus (60 mg/kg b.w.)**

*** The difference is significant compared to effect of urea**

**+ The difference is significant compared to effect of hydrochlorothiazide**

**T/U "Lipschitz-value" ratio in which T is the response of the tested compound and U is the urea response**

### *4.3.3* Natriuretic effects of the test compounds

The results of natriuretic effects of ursolic acid, oleanolic acid and the 16 phytochemicals are presented in tables 5a and 5b. All the test compounds did have some natriuretic effect on the rats at 5 hours after their administration. Analysis of the urine collected after 24 hours from the time the compounds were administered, showed that oleanolic acid, ursolic acid, *Psidium guajava* (small fruit), *P. guajava* (white fruit), *Psidium guajava* (hybrid), *Terminalia sericea, Prunus africana* and *Combretum molle* had no natriuretic effect even after 24 hours. *Psidium guajava* (pink fruit), *Plantago major, Clerodendrum L, Buddleja salviifolia, Tetradenia riparia* and *Centella asiatica* had a slight natriuretic effect. *Syzygum cordatum, Eugenia jambolana, Plantago lanceolata* and *Psychotreia serpens* had similar natriuretic effects to those of hydrochlorothiazide as their effects were not significantly different. Actually, *Eugenia jambolana* had better natriuretic effect than the hydrochlorothiazide.

## 4.4- EFFECTS ON BLOOD PRESSURE

The haemodynamic results of the pure triterpenoids isolated in this study, that is OA, UA, UV and MM, are presented in Table 6 and Figures 16 - 21. All the four triterpenoids showed a significant vasodepressor effect and sinus bradycardia that lasted for more than 60 min. These effects were dose-dependent with the peak effect seen at 20 min after application. The most potent effects were shown by OA and MM. Table 7 and Figures 22 and 23 shows the effect of isoprenaline after 15 minutes pre-treatment with the four triterpenoids and a propranolol, a beta-adrenergic blocker that acted as a reference drug. The pre-treatment with the triterpenoids decreased the vasopressor and tachycardic effects

of isoprenaline. Oleanolic acid had the most potent effect than the other three triterpenoids as its reduction of the vasopressor effect of isoprenaline was similar to that of the reference drug, propranolol.

The results of blood pressure and heart rate effects of the phytochemicals after a single intraperitoneal application (100 mg/ kg b.w) are presented in Table 8 and Figures 24a – 26b. All the phytochemicals showed a trend of decreased blood pressure on the 10 minutes after a single intraperitoneal application except those from *Clerodendrum capense*, *Prunus africana, Psidium guajava* (hybrid), *Terminalia sericea* and *Plantago lanceolata.* Of the plants that showed a decreased trend of blood pressure, only *Syzygum cordatum, Eugenia jambolana, Plantago major, Buddleja salviifolia, Tetradenia riparia, Psidium guajava* (pink fruit) and *Psychotreia serpens* had their effects persisting for more than 60 minutes. On the other hand, *Psidium guajava* (small fruit), *Psidium guajava* (white fruit), *Combretum molle* and *Centella asiatica* had their hypotensive effects lasting for less than 30 minutes. Only *Eugenia jambolana,* all the *Psidium guajava* subspecies, *Terminalia sericea and Tetradenia riparia* produced significant bradycardia activity in the animals after a single intraperitoneal injection of the phytochemicals.

Table 9 and Figures 27a – 29b shows the haemodynamic effects of the phytochemicals after six days daily intraperitoneal treatment (100 mg/kg b.w). The daily dose was applied after the routine every morning blood pressure measurements. All the phytochemicals save those from *Psidium guajava* (pink fruit), *Psidium guajava* (hybrid) and *Terminalia sericea* reduced blood pressure at one time or another. *Clerodendrum capense* reduced blood pressure every other day whilst *Syzygum cordatum* reduced blood pressure on the second day only. Plantago *major* reduced blood pressure on the 1$^{st}$, 5$^{th}$ and 6$^{th}$ days whilst *Prunus*

*africana, Combretum molle* and *Buddleja salviifolia* reduced blood pressure on the 5th and 6th days. *Plantago lanceolata* and *Centella asiatica* consistently reduced blood pressure from the third day onwards whilst *Eugenia jambolana, Tetradenia riparia* and *Psychotreia serpens* consistently reduced blood pressure right from the first day. Only *Syzygum cordatum, Psidium guajava* (pink fruit), *Clerodendrum capense, Plantago major, Plantago lanceolata, Combretum molle* and *Psychotreia serpens* did not have any bradycardia effect on the animals whilst the rest of the phytochemicals had this effect.

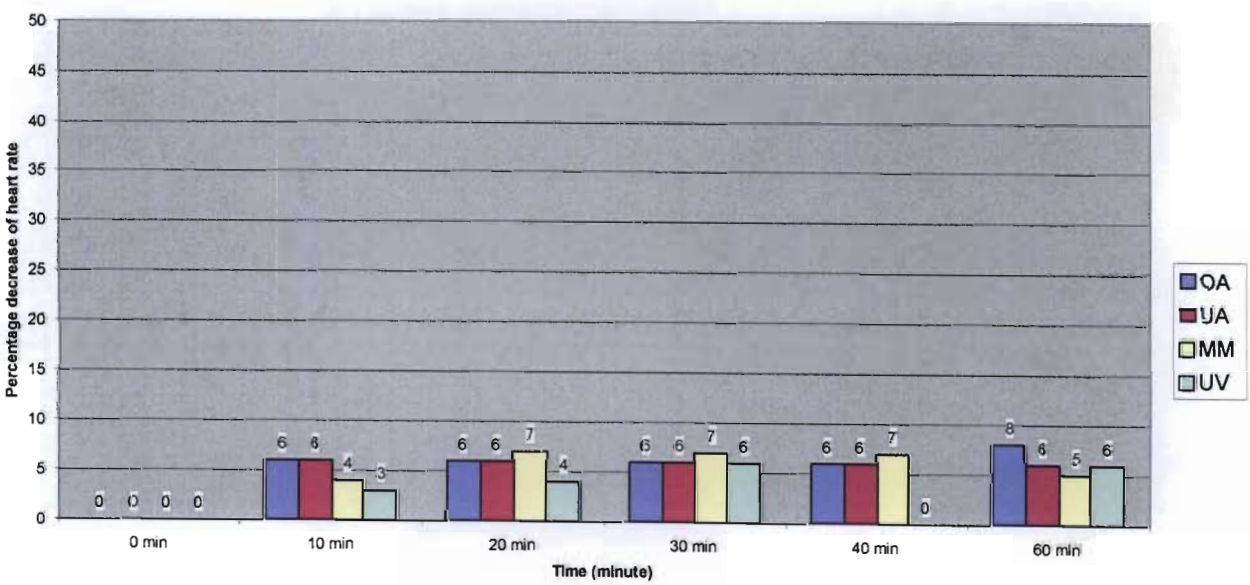


**Figure 16. Changes in heart rate after i.p (20 mg/kg) application of OA,UA, UV and MM**

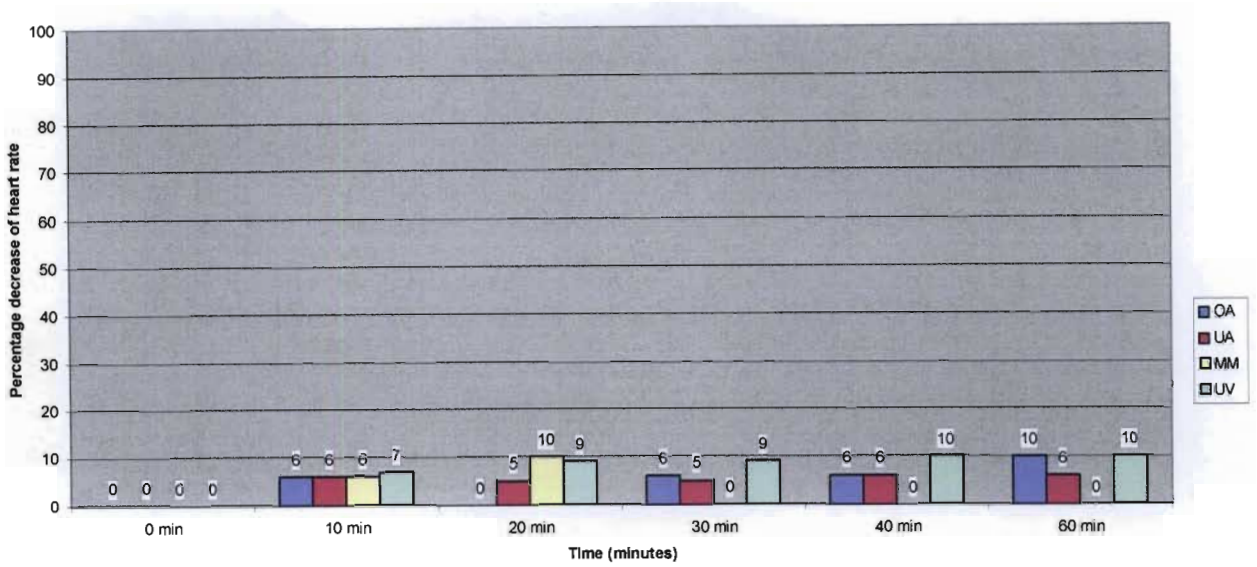


**Figure 17. Changes in heart rate after i.p (40 mg/kg) application of different doses of OA, UA, UV and MM**

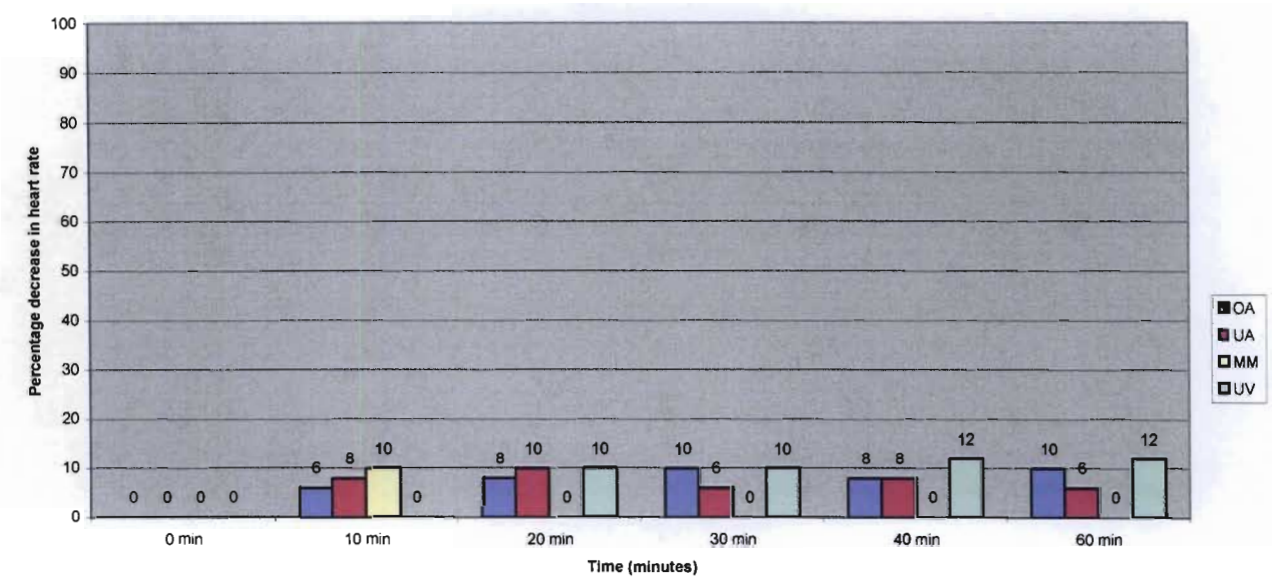


**Figure 18. Changes in heart rate after i.p(60 mg/kg) application of OA, UA, UV and MM**

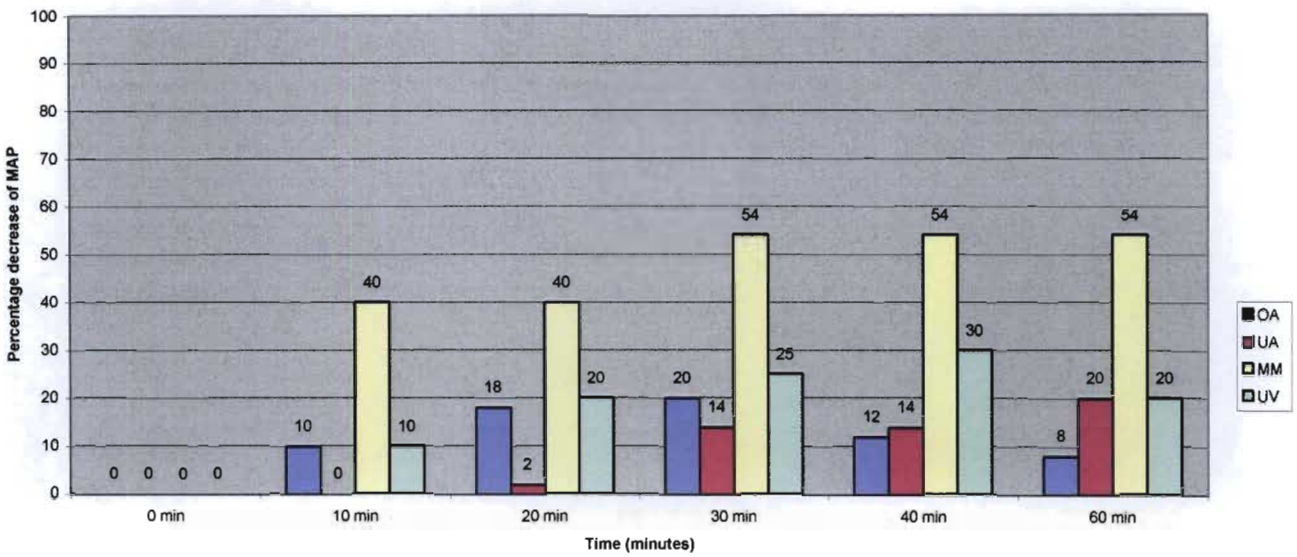


**Figure 19. Changes in mean arterial pressure (MAP) after i.p (20 mg/kg) application of OA, UA, UV and MM**

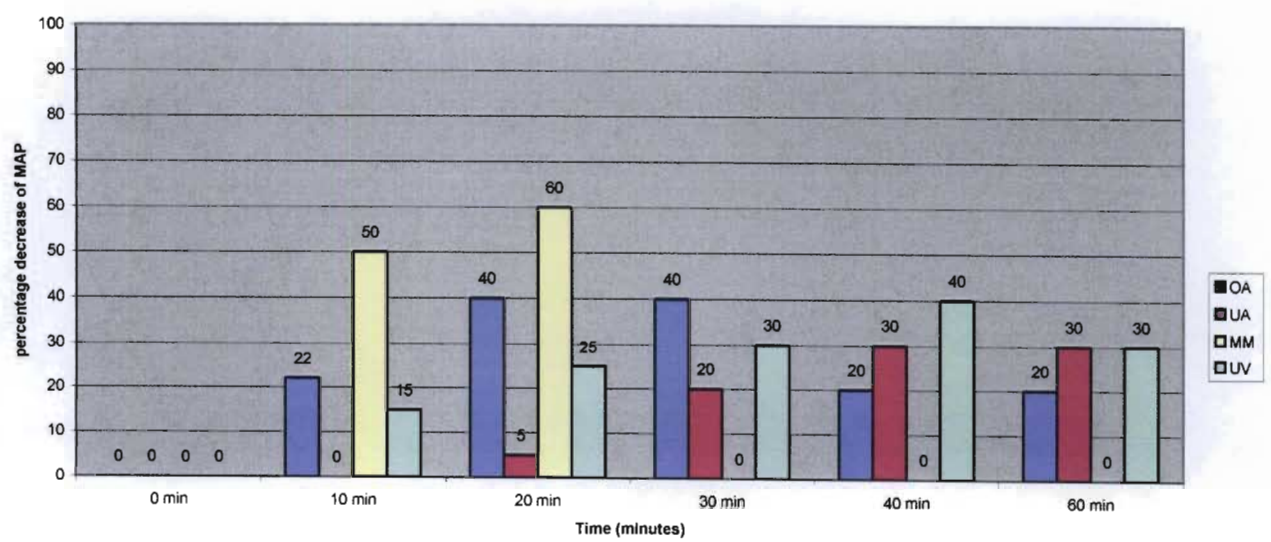


**Figure 20. Changes in mean arterial pressure (MAP) after i.p (40 mg/kg) application of OA, UA, UV and MM**

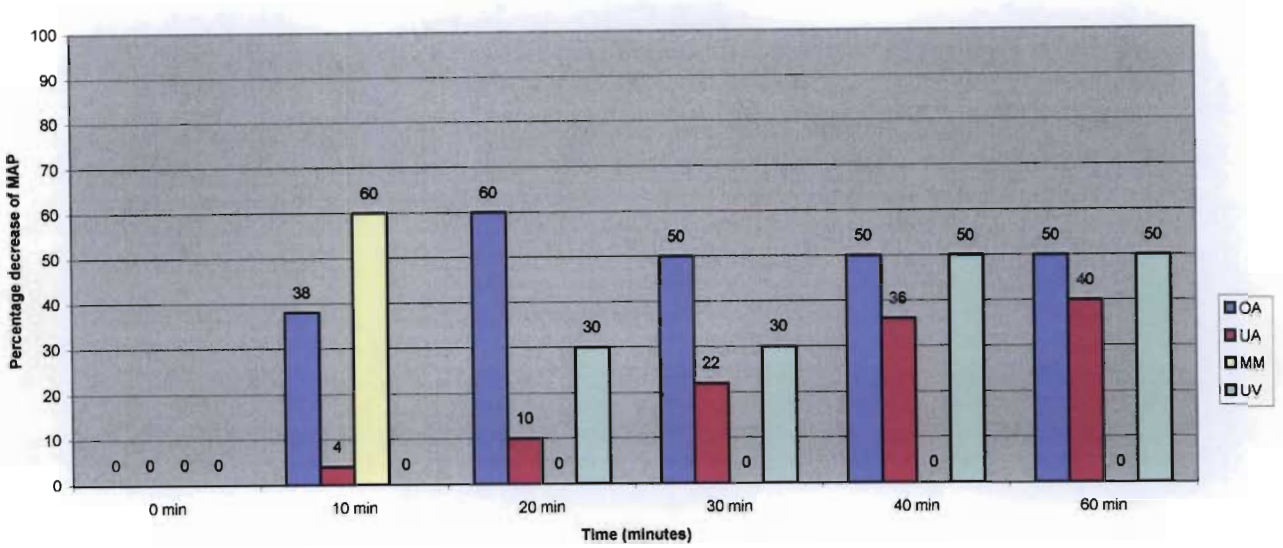


**Figure 21. Changes in mean arterial pressure (MAP) after i.p (60 mg/kg) application of OA, UA, UV and MM**

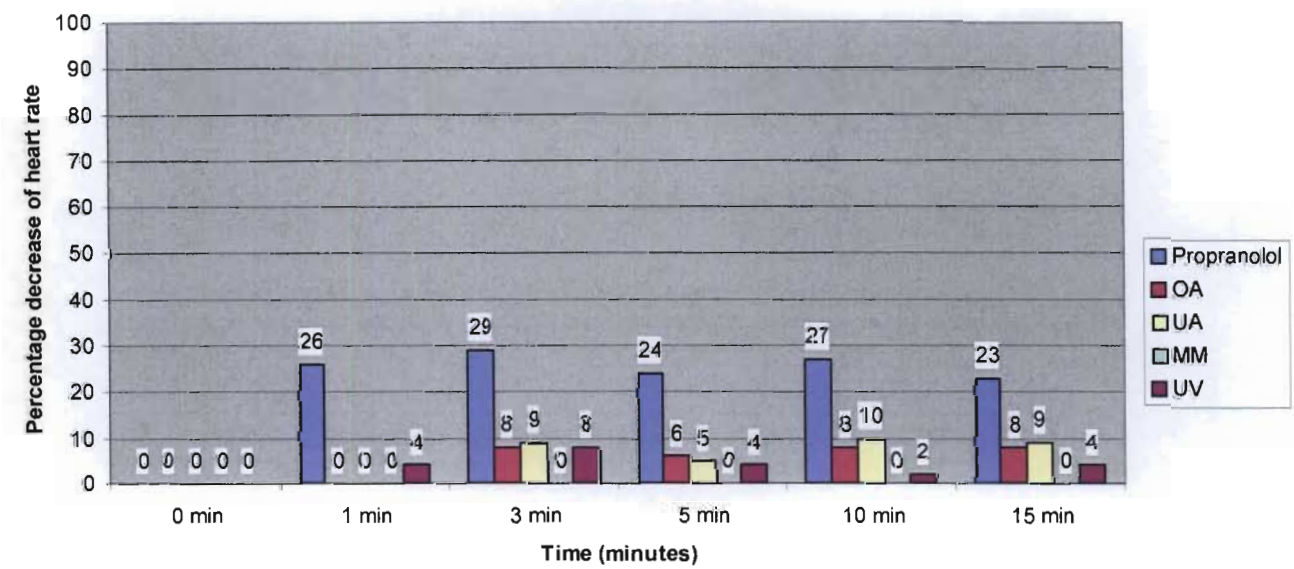


**Figure 22. Effects of isoprenaline (1 ug/kg) on heart rate after 15 minute pretreatment with reference substance (propranolol, 2mg/kg) and different triterpenoids (10 mg/kg)**

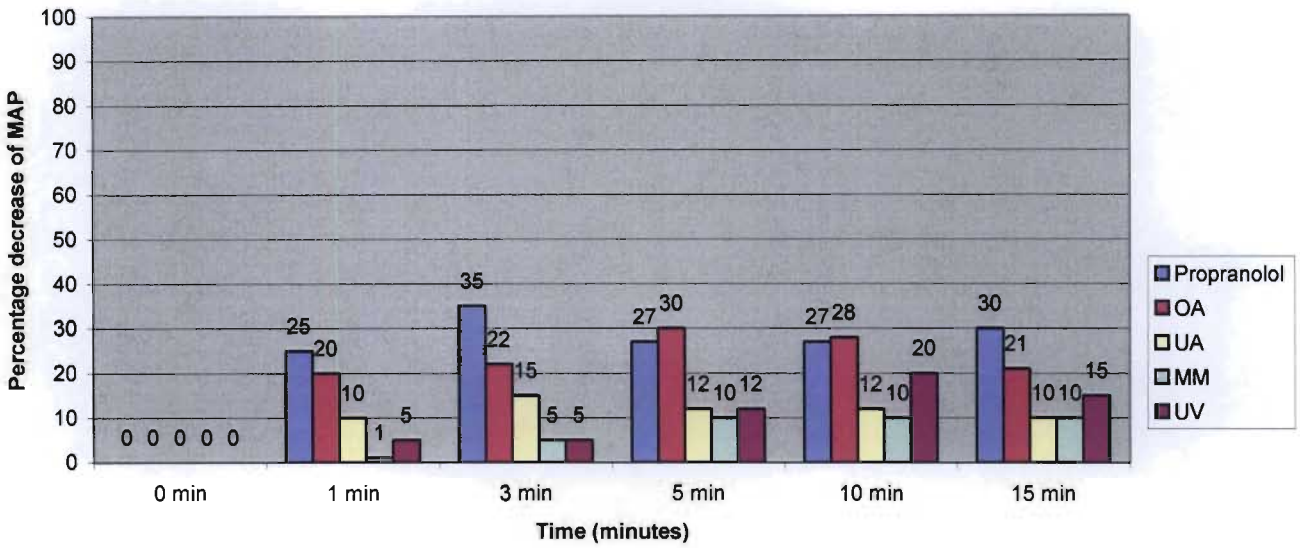


**Figure 23. Effects of isoprenaline (1 ug/kg) on mean arterial pressure (MAP) after 15 minutes pretreatment with reference substance (propranolol, 2 mg/kg) and different triterpenoids (10 mg/kg)**

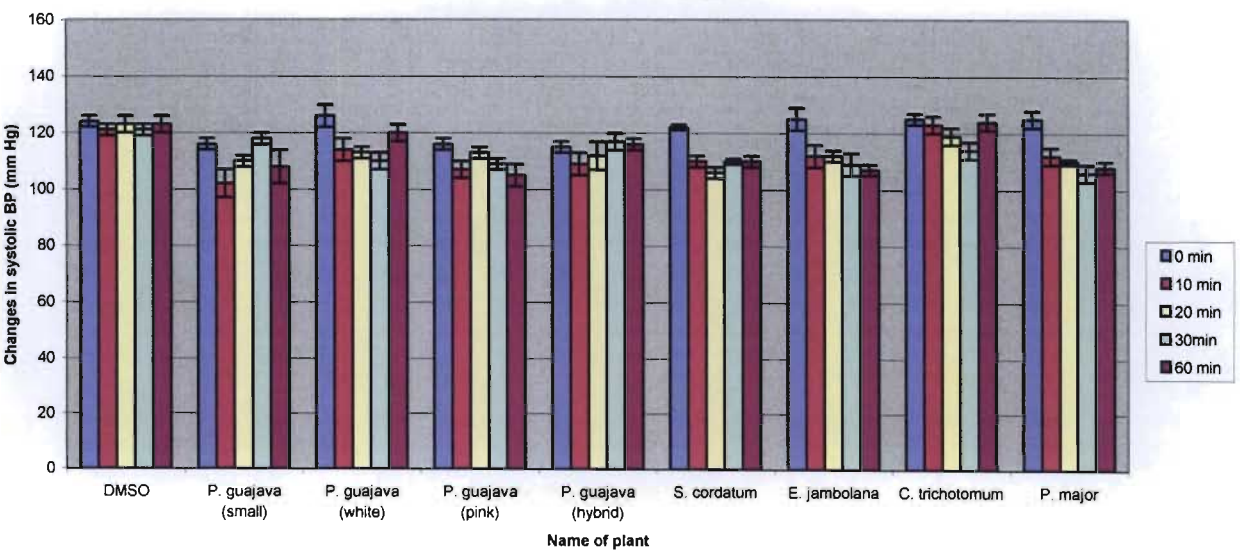


**Figure 24a. Follow-up changes in systolic blood pressure (mm Hg) after a single i.p. of phytochemical extractives (100 mg/kg b.w)**

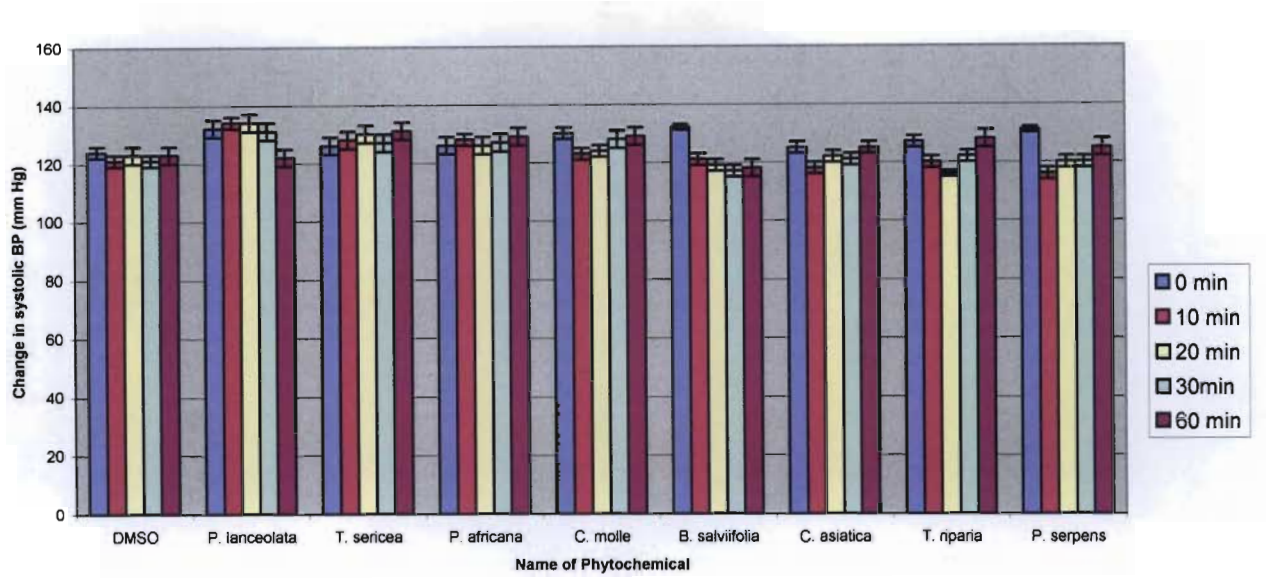


**Figure 24b. Follow-up changes in systolic blood pressure (mm Hg) in rats after a single i.p. injection of phytochemical extractives (100 mg/kg b.w)**

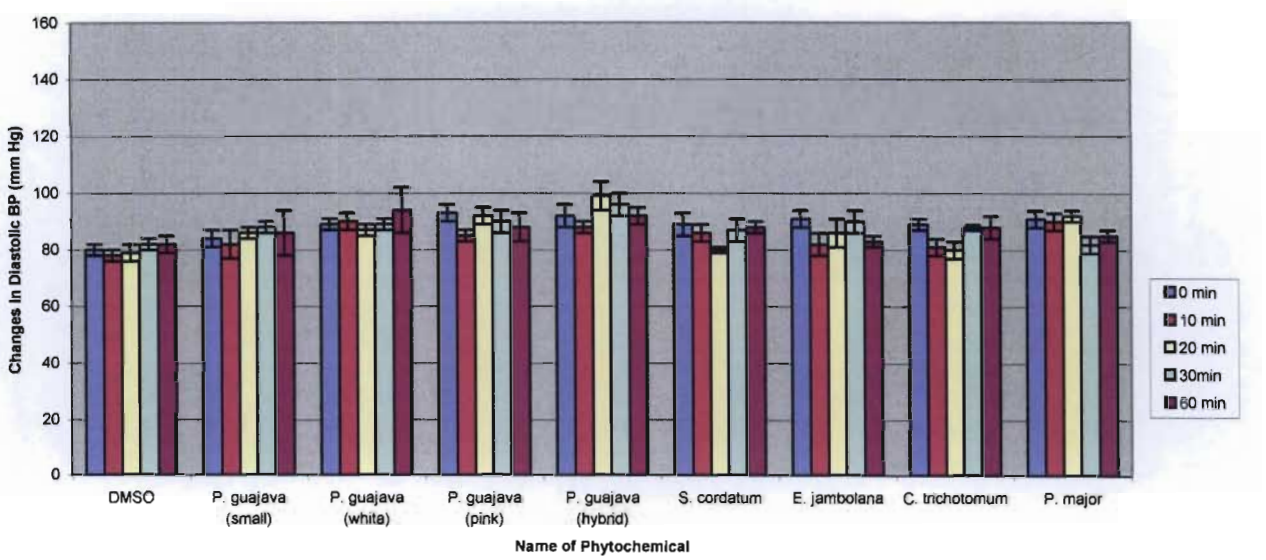


**Figure 25a. Follow-up changes in diastolic blood pressure (mm Hg) in rats after a single i.p. injection of phytochemical extractives (100 mg/kg b.w)**

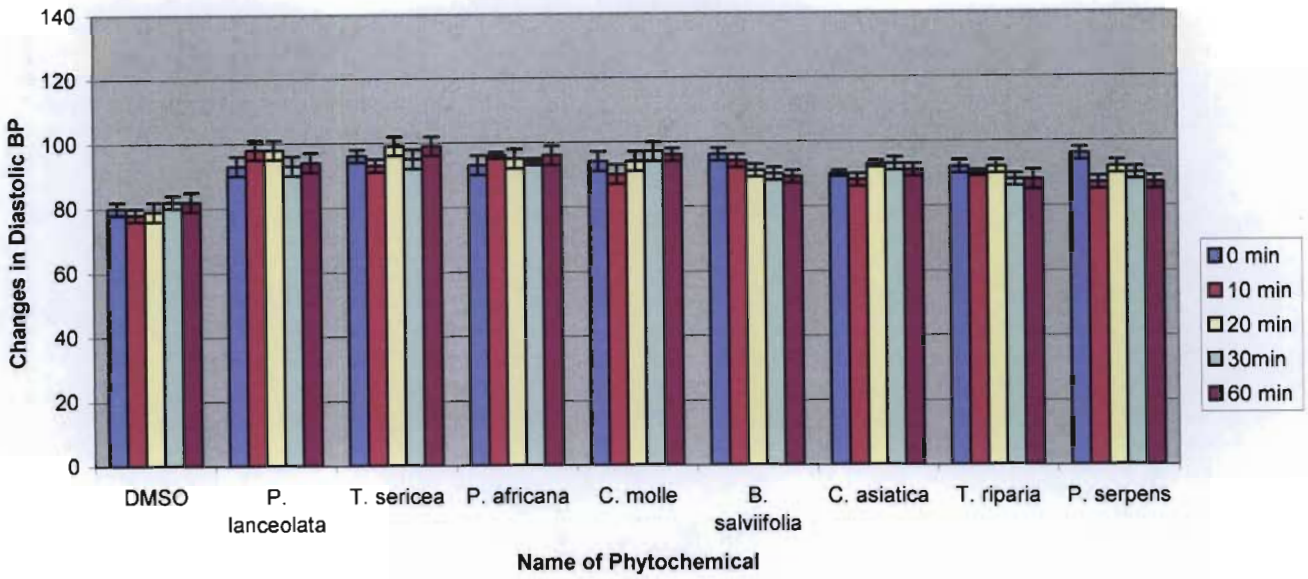


**Figure 25b. Follow-up changes in diastolic blood pressure (mm Hg) of rats after a single i.p. injection of the phytochemicals (100 mg/kg b.w)**

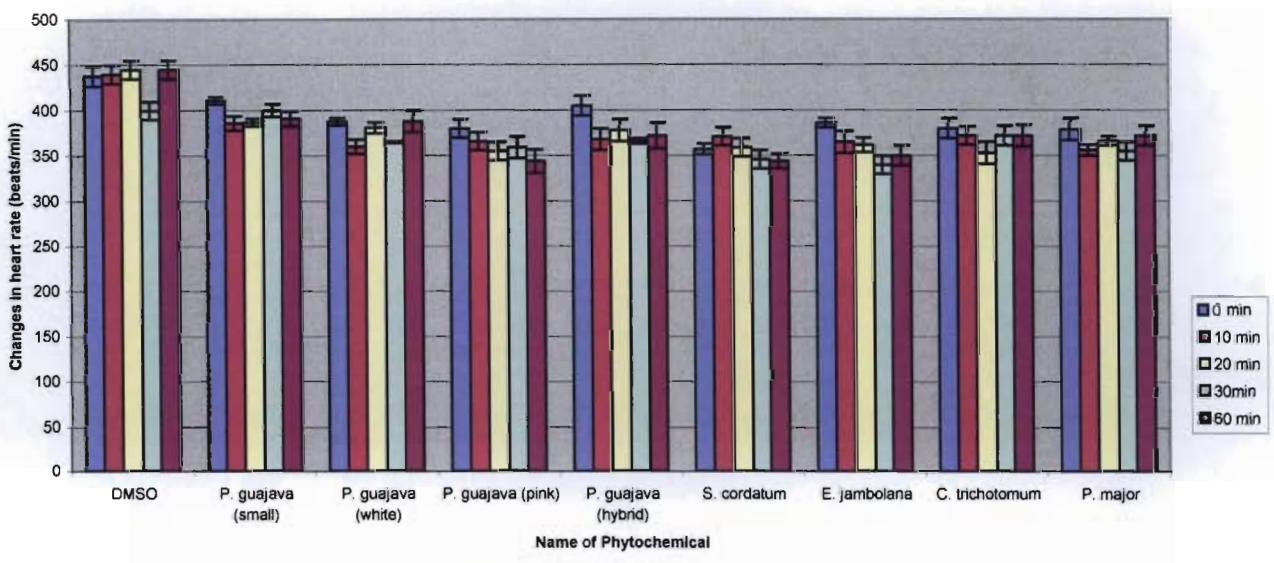


**Figure 26a. Follow-up changes in heart rate (beats/min) of wistar rats after a single i.p injection of the phytochemical extractive (100 mg/kg b.w)**

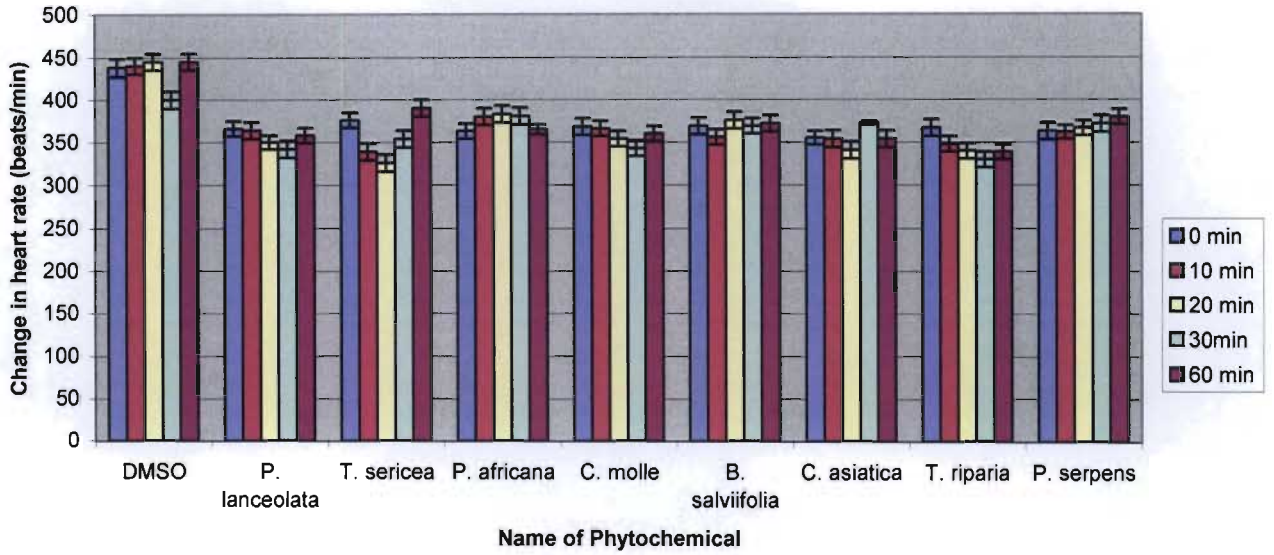


**Figure 26b. Follow-up changes in heart rate (beats/min) of Wistar rats after a single i.p. injection of the phytochemical extractives (100 mg/kg b.w)**

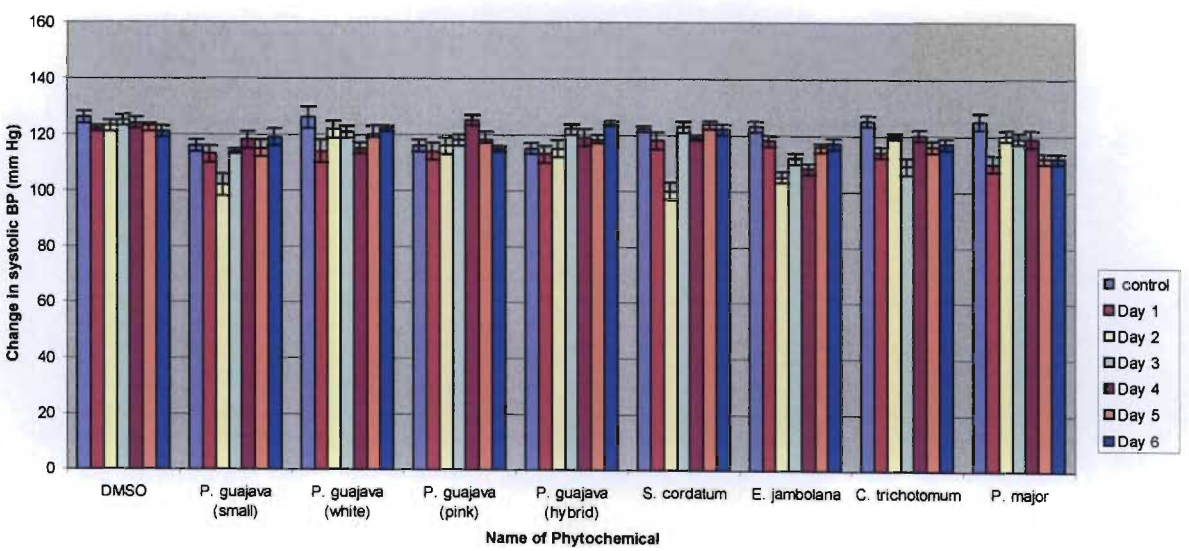


**Figure 27a. Changes in systolic blood pressure (mm Hg) of Wistar rats treated with the phytochemical extractives for 6 consecutive days**

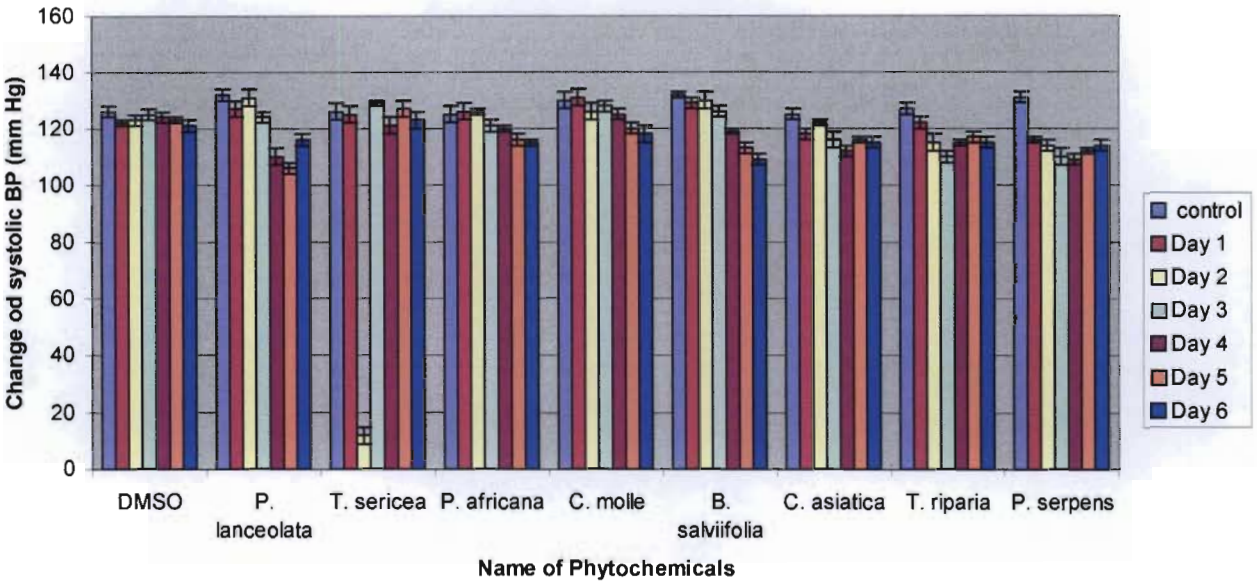


**Figure 27b. Changes in systolic blood pressure (mm Hg) of Wistar rats treated with the phytochemicals for 6 consecutive days**

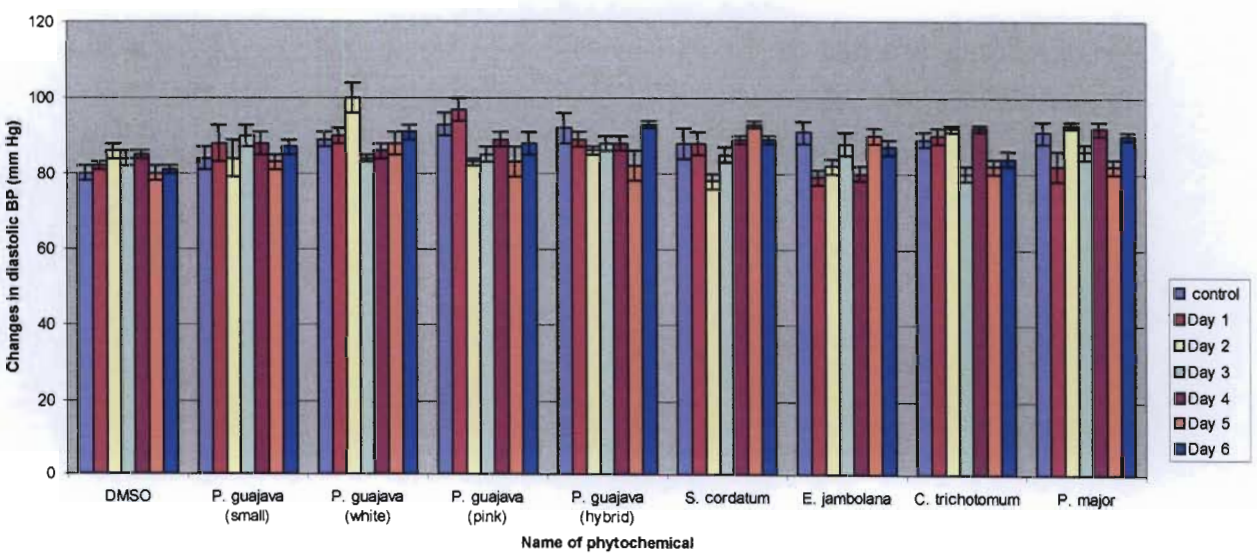


**Figure 28a. Changes in diastolic blood pressure (mm Hg) of Wistar rats treated with phytochemicals (100 mg/kg b.w) for 6 consecutive days**

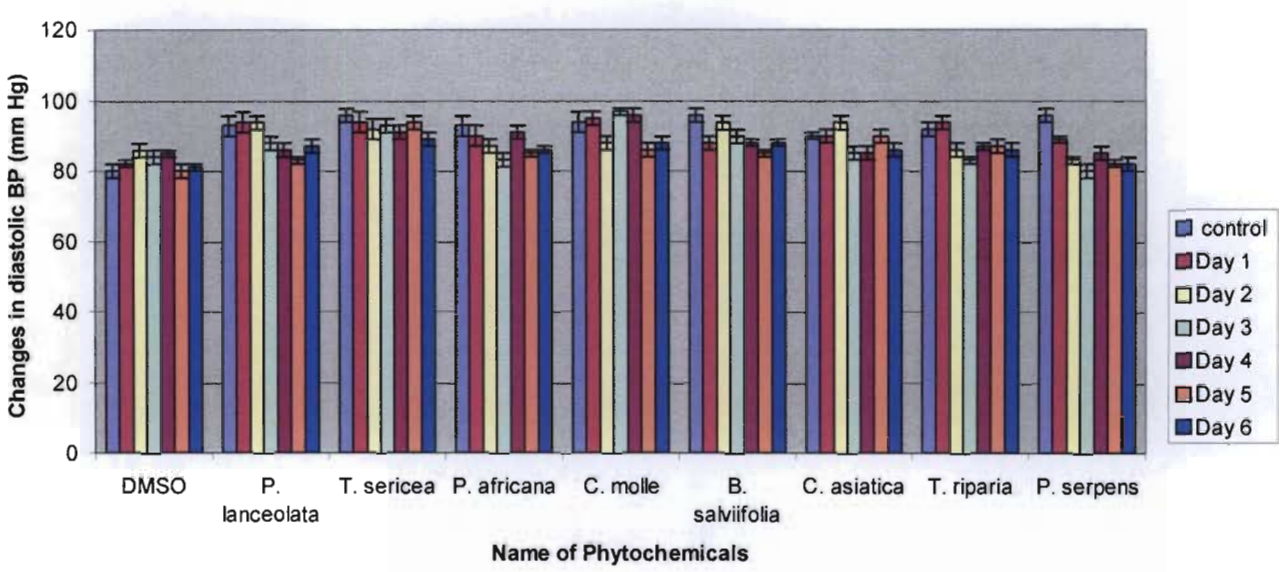


**Figure 28b. Changes in diastolic blood pressure (mm Hg) of Wistar rats treated with phytochemicals for 6 consecutive days**

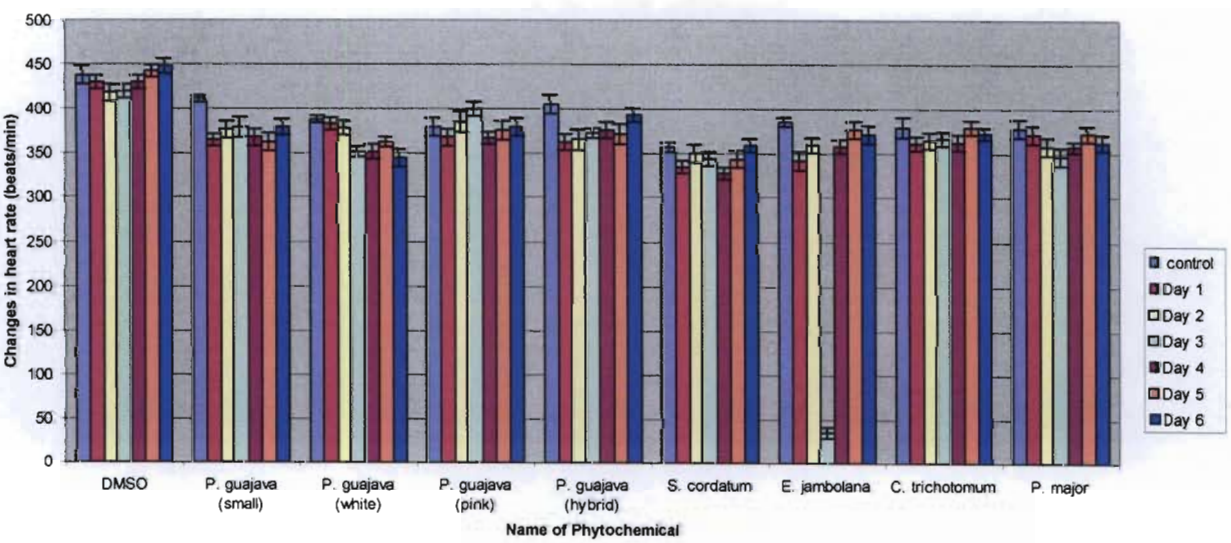


**Figure 29a. Changes in heart rate (beats/min) of Wistar rats treated with the phytochemical extractives for 6 consecutive days**

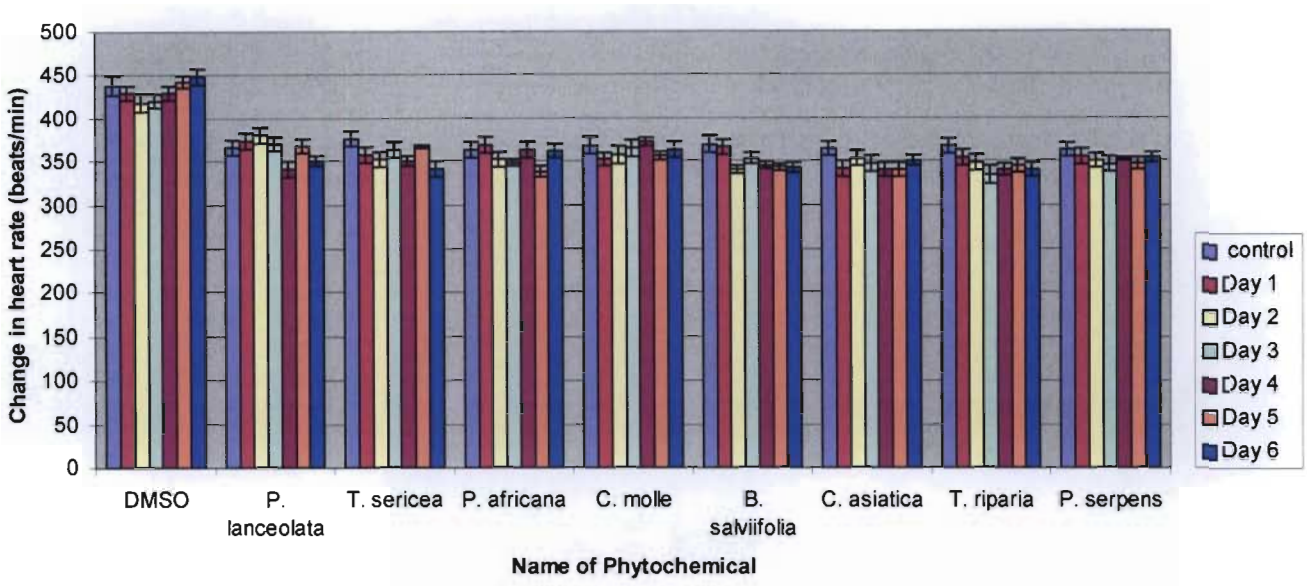


**Figure 29b. Changes in heart rate (beats/min) of Wistar rats treated with the phytochemical extractives (100 mg/kg b.w) for 6 consecutive days**

**Table 6: Changes in heart rate (HR) and mean arterial pressure (MAP) after intraperitoneal application of different doses of triterpenoids. The parameters were measured using the direct method**

| | | Baseline | | 10 minutes | | 20 minutes | | 30 minutes | | 40 minutes | | 60 minutes | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Group** | **Dose (mg/kg)** | **HR** | **MAP** | **HR** | **MAP** | **HR** | **MAP** | **HR** | **MAP** | **HR** | **MAP** | **HR** | **MAP** |
| **OA** | 20 | 495 ± 8 | 96 ± 6 | ↓6% | ↓10% | ↓6% | ↓18% | ↓6% | ↓20% | ↓6% | ↓12% | ↓8% | ↓8% |
| | 40 | 480 ±10 | 92 ± 4 | ↓6% | ↓22% | 0 | ↓40% | ↓6% | ↓40% | ↓6% | ↓20% | ↓10% | ↓20% |
| | 60 | 450 ± 8 | 104 ± 6 | ↓6% | ↓38% | ↓8% | ↓60% | ↓10% | ↓50% | ↓8% | ↓50% | ↓10% | ↓50% |
| **UA** | 20 | 480 ± 6 | 84 ± 4 | ↓6% | 0 | ↓6% | ↓2% | ↓6% | ↓14% | ↓6% | ↓14% | ↓6% | ↓20% |
| | 40 | 465 ± 6 | 96 ± 4 | ↓6% | 0 | ↓5% | ↓5% | ↓5% | ↓20% | ↓6% | ↓30% | ↓6% | ↓30% |
| | 60 | 480 ± 8 | 94 ± 6 | ↓8% | ↓4% | ↓10% | ↓10% | ↓6% | ↓22% | ↓8% | ↓36% | ↓6% | ↓40% |
| **MM** | 20 | 420 ± 8 | 110 ± 8 | ↓4% | ↓40% | ↓7% | ↓40% | ↓7% | ↓54% | ↓7% | ↓54% | ↓5% | ↓54% |
| | 40 | 400 ± 8 | 100 ± 10 | ↓6% | ↓50% | ↓10% | ↓60% | 0 | 0 | 0 | 0 | 0 | 0 |
| | 60 | 400 ± 6 | 115 ± 8 | ↓10% | ↓60% | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **UV** | 20 | 435 ± 7 | 98 ± 4 | ↓3% | ↓10% | ↓4% | ↓20% | ↓6% | ↓25% | 0 | ↓30% | ↓6% | ↓20% |
| | 40 | 430 ±10 | 100 ± 6 | ↓7% | ↓15% | ↓9% | ↓25% | ↓9% | ↓30% | ↓10% | ↓40% | ↓10% | ↓30% |
| | 60 | 420 ± 8 | 96 ± 6 | 0 | 0 | ↓10% | ↓30% | ↓10% | ↓30% | ↓12% | ↓50% | ↓12% | ↓50% |

**Mean** ± SEM; All changes are significant compared to control values ↓ - **Decrease**
**OA – Oleanolic acid UA - Ursolic acid MM- Methyl maslinate UV – Uvaol**
**HR – Heart rate (beats/min) MAP – Mean arterial pressure (mm Hg)**

**Table 7: Beta-adrenolitic effects of triterpenoids isolated from plants in wistar rats: Effects of isoprenaline (1 µg/kg) after 15 minutes pre-treatment with reference substance (propranolol) and different isolates**

| | Baseline | | 1 minute | | 3 minute | | 5 minute | | 10 minute | | 15 minute | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **TREATMENT** | **MAP** | **HR** | **MAP** | **HR** | **MAP** | **HR** | **MAP** | **HR** | **MAP** | **HR** | **MAP** | **HR** |
| Propranolol i.v (2 mg/kg) | 150 ± 4.2 | 450 ± 8.0 | ↓25% | ↓26% | ↓35% | ↓29% | ↓27% | ↓24% | ↓27% | ↓27% | ↓30% | ↓23% |
| Oleanolic acid i.p. (10 mg/kg) | 145 ± 6.0 | 462 ± 8.8 | ↓20% | ↓0% | ↓22% | ↓8% | ↓30% | ↓6% | ↓28% | ↓8% | ↓21% | ↓8% |
| Ursolic acid i.p (10 mg/kg) | 135 ± 4.0 | 440 ± 9.0 | ↓10% | 0 | ↓15% | ↓9% | ↓12% | ↓5% | ↓12% | ↓10% | ↓10% | ↓9% |
| Methyl maslinate acid i.p. (10 mg/kg) | 142 ± 6.6 | 410 ± 8.8 | ↓ 1% | ↓0% | ↓5% | 0 | ↓10% | 0 | ↓10% | 0 | ↓10% | 0 |
| Uvaol i.p. (10 mg/kg) | 148 ± 8.0 | 416 ±12.0 | ↓ 5% | ↓4% | ↓5% | ↓8% | ↓12% | ↓4% | ↓20% | ↓2% | ↓15% | ↓4% |

**Baseline values -** **Mean ± SEM**
**↓ -** **Percentage decrease compared to the original effect of isoprenaline before treatment**
**MAP -** **Mean arterial pressure (mm Hg)**
**HR -** **Heart rate (beats/minute)**

**Table 8: Follow-up changes in blood pressure (mm Hg) and heart rate (beats/min) of Wistar rats after a single intraperitoneal injection of the phytochemical extractives (100 mg/ kg b.w) using the cuff tail method**

| | Baseline | | | 10 minutes | | | 20 minutes | | | 30 minutes | | | 60 minutes | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Group/Parameter** | **SBP** | **DBP** | **HR** | **SBP** | **DBP** | **HR** | **SBP** | **DBP** | **HR** | **SBP** | **DBP** | **HR** | **SBP** | **DBP** | **HR** |
| Vehicle (DMSO) | 124 ± 2 | 80 ± 2 | 438 ±11 | 121 ± 2 | 78 ± 2 | 440 ±10 | 123 ± 3 | 79 ± 3 | 445 ±10 | 121 ± 2 | 82 ± 2 | 400 ±10 | 123 ± 3 | 82 ± 3 | 445 ±10 |
| *Psidium guajava* (small) | 116 ± 2 | 84 ± 3 | 411 ± 4 | 102 ± 5* | 82 ± 5 | 386 ±8* | 110 ±2* | 86 ± 2 | 387 ±4* | 118 ± 2 | 88 ± 2 | 400 ± 7 | 108 ± 6 | 86 ± 8 | 391 ± 8 |
| *Psidium guajava* (white) | 126 ± 4 | 89 ± 2 | 388 ± 4 | 114 ± 4* | 90 ± 3 | 360 ±8* | 113 ±2* | 87 ± 2 | 381 ± 6 | 110 ± 3* | 89 ± 2 | 365 ±10 | 120 ± 3 | 94 ± 8 | 388 ±12 |
| *Psidium guajava* (pink) | 116 ± 2 | 93 ± 3 | 380 ±10 | 107 ± 3* | 85 ± 2* | 366 ±10 | 113 ± 2 | 92 ± 3 | 355 ±11 | 109 ± 2* | 90 ± 4 | 359 ±12 | 105 ±4* | 88 ± 5 | 344 ±13* |
| *Psidium guajava* (hybrid) | 115 ± 2 | 92 ± 4 | 405 ±11 | 109 ± 4 | 88 ± 2 | 368 ±12* | 112 ± 5 | 99 ± 5 | 378 ±12 | 117 ± 3 | 96 ± 4 | 366 ±3* | 116 ± 2 | 92 ± 3 | 372 ±14 |
| *Syzygum cordatum* | 122 ± 1 | 89 ± 4 | 357 ± 6 | 110 ± 2* | 86 ± 3 | 371 ±10 | 106 ±2* | 80 ± 1* | 359 ±10 | 110 ± 1* | 87 ± 4 | 346 ±10 | 110 ±2* | 88 ± 2 | 344 ± 8 |
| *Eugenia jambulana* | 125 ± 4 | 91 ± 3 | 386 ± 5 | 112 ± 4* | 82 ± 4 | 365 ±12 | 112 ±2* | 86 ± 5 | 362 ±8* | 109 ± 4* | 90 ± 4 | 340 ±10* | 107 ±2* | 83 ±2* | 350 ±11* |
| *Clerodendrum capense* | 125 ± 2 | 89 ± 2 | 380 ±11 | 123 ± 3 | 81 ± 3 | 372 ±11 | 119 ± 3 | 80 ± 3 | 353 ±12 | 114 ± 3 | 88 ± 1 | 372 ±11 | 124 ± 3 | 88 ± 4 | 372 ±12 |
| *Plantago major* | 125 ± 3 | 91 ± 3 | 379 ±12 | 112 ± 3* | 90 ± 3 | 356 ± 6 | 110 ±1* | 92 ± 2 | 366 ± 5 | 106 ± 3* | 82 ± 3* | 355 ±10 | 108 ±2* | 85 ± 2 | 372 ±11 |
| *Plantago lanceolata* | 132 ± 3 | 93 ± 3 | 366 ± 9 | 134 ± 2 | 98 ± 3 | 364 ±10 | 134 ± 3 | 98 ± 3 | 350 ± 8 | 131 ± 3 | 93 ± 3 | 342 ±10 | 122 ±3* | 94 ± 3 | 358 ± 9 |
| *Terminalia sericea* | 126 ± 3 | 96 ± 2 | 376 ± 9 | 128 ± 3 | 93 ± 2 | 339 ±10 | 130 ± 3 | 99 ± 3 | 326 ±10* | 127 ± 3 | 95 ± 3 | 354 ±10 | 131 ± 3 | 99 ± 3 | 390 ±10 |
| *Prunus africana* | 126 ± 3 | 93 ± 3 | 363 ± 9 | 128 ± 2 | 96 ± 1 | 380 ±10 | 126 ± 3 | 95 ± 3 | 383 ± 10 | 127 ± 3 | 94 ± 1 | 381 ±10 | 129 ± 3 | 96 ± 3 | 365 ± 6 |
| *Combretum molle* | 130 ± 2 | 94 ± 3 | 368 ±10 | 123 ± 2* | 90 ± 3 | 366 ± 9 | 124 ±2* | 94 ± 3 | 354 ± 9 | 128 ± 3 | 97 ± 3 | 343 ± 9 | 129 ± 3 | 96 ± 2 | 365 ± 9 |
| *Buddleja salvifolia* | 132 ± 1 | 96 ± 2 | 369 ±10 | 121 ± 2* | 94 ± 2 | 356 ± 9 | 119 ±2* | 91 ± 2 | 376 ± 10 | 117 ± 2* | 90 ± 2 | 369 ± 9 | 118 ±3* | 89 ±2* | 372 ±10 |
| *Centella asiatica* | 125 ± 2 | 90 ± 1 | 355 ± 8 | 118 ± 2* | 88 ± 2 | 354 ±10 | 122 ± 2 | 93 ± 1 | 341 ± 10 | 121 ± 2 | 93 ± 2 | 373 ± 2 | 125 ± 2 | 91 ± 2 | 354 ±10 |
| *Tetradenia riparia* | 127 ± 2 | 92 ± 2 | 367 ±10 | 120 ± 2* | 90 ± 1 | 348 ± 9 | 116 ±1* | 92 ± 2 | 340 ± 9* | 122 ± 2 | 88 ± 2 | 330 ±9* | 128 ± 3 | 88 ± 3 | 339 ±9* |
| *Psychotreia serpeus* | 131 ± 2 | 96 ± 2 | 363 ±10 | 116 ± 2* | 87 ± 2* | 362 ± 8 | 120 ±2* | 92 ± 2 | 367 ± 9 | 120 ± 2* | 90 ± 2* | 372 ±10 | 125 ± 3 | 87 ±2* | 380 ± 9 |

**Mean ± SEM; SBP – Systolic Blood Pressure; DPB – Diastolic Blood Pressure; HR – Heart Rate * Significant compared to baseline value**
**DMSO – Dimethyl sulfoxide The phytochemicals were applied intraperitoneally in a dose 100 mg/kg b.w. except for Psychotreia serpeus and DMSO (60 mg/kg b.w.)**

**Table 9: Changes in blood pressure (mm Hg) and heart rate (beats/ min) of Wistar rats treated with the phytochemical extractives for six consecutive days**

| | Control | | | 1 day | | | 2 days | | | 3 days | | | 4 days | | | 5 days | | | 6 days | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Group/ Parameter** | **SBP** | **DBP** | **HR** | **SBP** | **DBP** | **HR** | **SBP** | **DBP** | **HR** | **SBP** | **DBP** | **HR** | **SBP** | **DBP** | **HR** | **SBP** | **DBP** | **HR** | **SBP** | **DBP** | **HR** |
| Vehicle (DMSO) | 126 ± 2 | 80 ± 2 | 438 ±11 | 122 ± 1 | 82 ± 1 | 430 ± 8 | 123 ± 2 | 86 ± 2 | 418 ±10 | 125 ± 2 | 84 ± 2 | 420 ± 8 | 124 ± 2 | 85 ± 1 | 430 ± 8 | 123 ± 1 | 80 ± 2 | 442 ± 7 | 121 ± 2 | 81 ± 1 | 448 ± 9 |
| *P. guajava* (small) | 116 ± 2 | 84 ± 3 | 411 ± 4 | 113 ± 3 | 88 ± 5 | 365 ±7* | 102 ± 4* | 84 ± 5 | 377 ±10* | 114 ± 1 | 90 ± 3 | 380 ±11 | 118 ± 3 | 88 ± 3 | 368 ±10 | 115 ± 3 | 83 ± 2 | 363 ±10* | 119 ± 3 | 87 ± 2 | 380 ±9* |
| *P. guajava* (white) | 126 ± 4 | 89 ± 2 | 388 ± 4 | 114 ± 4* | 90 ± 2 | 383 ± 7 | 122 ± 3 | 100 ± 4 | 379 ± 8 | 121 ± 2 | 84 ±1* | 352 ±6* | 115 ± 2* | 86 ± 2 | 352 ±9* | 120 ± 3 | 88 ± 3 | 363 ± 6* | 122 ± 1 | 91 ± 2 | 345 ±10* |
| *P. guajava* (pink) | 116 ± 2 | 93 ± 3 | 380 ±10 | 114 ± 3 | 97 ± 3 | 368 ±10 | 116 ± 3 | 83 ±1* | 386 ±12 | 118 ± 2 | 85 ± 2 | 400 ± 8 | 125 ± 2 | 89 ± 2 | 367 ± 7 | 118 ± 3 | 83 ± 4 | 376 ± 11 | 115 ± 1 | 88 ± 3 | 380 ± 10 |
| *P. guajava* (hybrid) | 115 ± 2 | 92 ± 4 | 405 ±11 | 113 ± 3 | 89 ± 2 | 363 ± 9 | 115 ± 3 | 86 ± 1 | 366 ±12* | 122 ± 2 | 88 ± 2 | 373 ±6* | 119 ± 3 | 88 ± 2 | 376 ± 9 | 118 ± 2 | 82 ± 4 | 372 ±11* | 124 ± 1 | 93 ± 1 | 393 ± 8 |
| *Syzygum cordatum* | 122 ± 1 | 88 ± 4 | 357 ± 6 | 118 ± 3 | 88 ± 3 | 335 ± 7 | 100 ±3* | 78 ±2* | 350 ± 10 | 123 ± 2 | 85 ± 2 | 345 ± 8 | 119 ± 1 | 89 ± 1 | 328 ± 7 | 124 ± 1 | 93 ± 1 | 344 ± 10 | 122 ± 2 | 89 ± 1 | 359 ± 8 |
| *Eugenia jambulana* | 123 ± 2 | 91 ± 3 | 386 ± 5 | 118 ± 2 | 79 ± 2 | 342 ±11* | 105 ±2* | 82 ±2* | 360 ± 8* | 112 ± 2* | 88 ± 3 | 369 ± 6 | 108 ± 2* | 80 ± 2* | 358 ±8* | 116 ± 1* | 90 ± 2 | 377 ± 10 | 117 ± 2* | 87 ± 2 | 372 ± 10 |
| *Clerodendru trichotomum* | 125 ± 2 | 89 ± 2 | 380 ±11 | 114 ±2* | 90 ± 2 | 362 ± 8 | 120 ± 1 | 92 ± 1 | 365 ± 9 | 109 ± 3* | 80 ± 2 | 367 ± 8 | 120 ± 2 | 92 ± 1 | 363 ± 9 | 116 ± 2* | 82 ± 2* | 380 ± 8 | 117 ± 2 | 84 ± 2 | 373 ± 7 |
| *Plantago major* | 125 ± 3 | 91 ± 3 | 379 ±10 | 110 ±3* | 82 ± 4* | 372 ±10 | 120 ± 2 | 93 ± 1 | 358 ± 10 | 119 ± 2 | 86 ± 2 | 347 ±10 | 119 ± 3 | 92 ± 2 | 358 ± 6 | 112 ± 2* | 82 ± 2* | 373 ± 9 | 112 ± 2* | 90 ± 1 | 363 ± 9 |
| *Plantago lanceolata* | 132 ± 2 | 93 ± 3 | 366 ± 9 | 127 ± 3 | 94 ± 3 | 373 ± 9 | 131 ± 3 | 94 ± 2 | 380 ± 9 | 124 ± 2* | 88 ± 2 | 370 ± 8 | 110 ± 3* | 86 ± 2 | 341 ± 9 | 106 ± 2* | 83 ± 1* | 368 ± 8 | 116 ± 2* | 87 ± 2 | 350 ± 6 |
| *Terminalia sericea* | 126 ± 3 | 96 ± 2 | 376 ± 9 | 125 ± 3 | 94 ± 3 | 357 ± 9 | 128 ± 3 | 92 ± 3 | 352 ± 9* | 129 ± 1 | 93 ± 2 | 363 ± 9 | 121 ± 3 | 91 ±2 | 350 ±6* | 127 ± 3 | 94 ± 2 | 367 ± 2 | 123 ± 3 | 89 ± 2 | 341 ± 9* |
| *Prunus africana* | 125 ± 3 | 93 ± 3 | 363 ± 9 | 126 ± 3 | 90 ± 3 | 369 ± 9 | 126 ± 1 | 87 ± 2 | 352 ± 9 | 121 ± 2 | 83 ±2* | 348 ± 4 | 120 ± 1 | 91 ± 2 | 363 ± 9 | 116 ± 2* | 85 ± 1* | 338 ± 6* | 115 ± 1* | 86 ± 1* | 362 ± 8 |
| *Combretum molle* | 130 ± 3 | 94 ± 3 | 368 ±10 | 131 ± 3 | 95 ± 2 | 352 ± 8 | 126 ± 3 | 88 ± 2 | 357 ±10 | 128 ± 2 | 97 ± 1 | 365 ±10 | 125 ± 2 | 96 ± 2 | 372 ± 5 | 120 ± 2* | 86 ± 2* | 356 ± 5 | 118 ± 3* | 88 ± 2 | 363 ± 9 |
| *Buddleja salvifolia* | 132 ± 1 | 96 ± 2 | 369 ±10 | 129 ± 2 | 88 ± 2* | 366 ± 9 | 130 ± 3 | 94 ± 2 | 340 ± 5 | 126 ± 2 | 90 ±2 | 353 ± 6 | 119 ± 1* | 88 ±1* | 345 ±4* | 113 ± 2* | 85 ± 1* | 343 ± 4* | 109 ± 2* | 88 ± 1* | 342 ± 6* |
| *Centella asiatica* | 125 ± 2 | 90 ± 1 | 364 ± 8 | 118 ±2* | 90 ± 2 | 341 ± 9 | 122 ± 1 | 94 ± 2 | 353 ± 9 | 116 ± 3* | 85 ±2 | 347 ± 9 | 112 ± 2* | 85 ±2 | 340 ±8* | 116 ± 1* | 90 ± 2 | 340 ± 8* | 115 ± 2* | 86 ± 2 | 350 ± 6 |
| *Tetradenia riparia* | 127 ± 2 | 92 ± 2 | 367 ± 9 | 122 ± 2 | 94 ± 2 | 354 ± 9 | 115 ± 3* | 86 ± 2 | 348 ± 9 | 110 ± 2* | 83 ±1* | 334 ±10* | 115 ± 1* | 87 ± 1 | 340 ±7* | 117 ± 2* | 87 ± 2 | 344 ± 8* | 115 ± 2* | 86 ± 2 | 340 ± 8* |
| *Psychotreia serpeus* | 131 ± 2 | 96 ± 2 | 363 ± 8 | 116 ±1* | 89 ± 1 | 355 ± 9 | 114 ±2* | 83 ±1* | 350 ± 8* | 110 ± 3* | 80 ±2* | 346 ± 9 | 109 ± 2* | 85 ±2* | 352 ± 2 | 112 ± 1* | 82 ± 1* | 347 ± 7 | 114 ± 2* | 82 ± 2* | 354 ± 6 |

**Mean ± SEM; SBP – Systolic Blood Pressure; DPB – Diastolic Blood Pressure; HR – Heart Rate * Significant compared to baseline value**

**The phytochemicals were applied intraperitoneally in a dose 100 mg/kg b.w. except for Psychotreia serpeus and DMSO (60 mg/kg b.w.)**

## 4.5 EFFECTS OF PHYTOCHEMICALS ON BODY WEIGHT

A few of the phytochemicals caused a significant decrease in the body weights of the animals when the test compounds were administered for six days (Table 10). The most pronounced decrease was seen in the rats that were injected with *Syzygum cordatum*. In these animals, the body weights were reduced from the second day up to fifth day, and from the sixth day the weight started to pick up again. *Psidium guajava* (hybrid) decreased the body weight from day 4 onwards whilst *Plantago lanceolata* caused a significant decrease in weight from day 5. No side or toxic effects were noticed during the 6 consecutive days of phytochemical administration.

## 4.6 ACUTE EFFECTS OF PHYTOCHEMICALS ON BLOOD GLUCOSE LEVELS

Blood glucose was estimated by Glucometer Elite, Bayer Diagnostics, in the whole blood collected from the rat's tail tip. The blood glucose levels were estimated before and 20 minutes after acute treatment with the phytochemicals. The results of the blood glucose levels are presented in table 11. Of all the test compounds, phytochemicals from *Psidium guajava* (small fruit) *Psidium guajava* (white fruit), *Clerodendrum capense, Terminalia sericea* and *Tetradenia riparia* displayed a potent hypoglycaemic activity. The most potent activity of these plants was that of Psidium guajava (white fruit).

**Table 10: Changes in body weight mass (g) of Wistar rats treated with phytochemicals for six consecutive days**

| Group/ Parameter | Control | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 | Day 6 |
|---|---|---|---|---|---|---|---|
| *Psidium guajava* (small) | 210 ± 4 | 211 ± 5 | 210 ± 5 | 211 ± 5 | 210 ± 4 | 211 ± 3 | 214 ± 3 |
| *Psidium guajava* (white) | 206 ± 2 | 201 ± 3 | 199 ± 4 | 197 ± 4* | 203 ± 4 | 203 ± 4 | 208 ± 4 |
| *Psidium guajava* (pink) | 260 ± 5 | 259 ± 5 | 260 ± 6 | 253 ± 6 | 246 ± 7 | 239 ± 6 | 248 ± 8 |
| *Psidium guajava* (hybrid) | 236 ± 5 | 228 ± 5 | 228 ± 6 | 222 ± 6 | 216 ± 7* | 213 ± 8* | 212 ± 8* |
| *Syzygum cordatum* | 230 ± 5 | 220 ± 7 | 208 ± 6* | 210 ± 6* | 210 ± 6* | 211 ± 8* | 216 ± 8 |
| *Eugenia jambulana* | 245 ± 5 | 237 ± 5 | 237 ± 5 | 230 ± 5 | 230 ± 6 | 229 ± 5* | 236 ± 7 |
| *Clerodendrum trichotomum* | 208 ± 5 | 202 ± 6 | 198 ± 4 | 203 ± 5 | 203 ± 5 | 203 ± 6 | 206 ± 6 |
| *Plantago major* | 216 ± 6 | 214 ± 5 | 213 ± 5 | 211 ± 6 | 214 ± 5 | 215 ± 4 | 214 ± 3 |
| *Plantago lanceolata* | 233 ± 4 | 239 ± 3 | 242 ± 4 | 242 ± 5 | 242 ± 4 | 250 ± 6* | 254 ± 8* |
| *Terminalia sericea* | 226 ± 6 | 229 ± 6 | 232 ± 7 | 233 ± 7 | 233 ± 6 | 237 ± 6 | 243 ± 6* |
| *Prunus africana* | 274 ± 9 | 271 ± 8 | 270 ± 9 | 265 ± 9 | 265 ± 9 | 267 ± 9 | 267 ± 9 |
| *Combretum molle* | 232 ± 8 | 230 ± 9 | 233 ± 9 | 234 ± 9 | 234 ± 8 | 240 ± 8 | 232 ± 7 |
| *Buddleja salvifolia* | 258 ± 9 | 259 ± 9 | 257 ± 9 | 263 ± 9 | 263 ± 8 | 266 ± 7 | 269 ± 6 |
| *Centella asiatica* | 255 ± 8 | 252 ± 6 | 257 ± 7 | 261 ± 8 | 261 ± 7 | 260 ± 7 | 262 ± 6 |
| *Tetradenia riparia* | 238 ± 8 | 236 ± 8 | 239 ± 8 | 242 ± 9 | 242 ± 9 | 239 ± 6 | 243 ± 6 |
| *Psychotreia serpeus* | 238 ± 4 | 232 ± 3 | 236 ± 3 | 236 ±3 | 236 ± 4 | 237 ± 5 | 243 ± 7 |

**Mean ± SEM**

*** The difference is significant compared to the control value**

**Table 11. Blood glucose levels in wistar rats before and 20 minutes after i.p. application of phytochemicals**

| Parameter | Glucose level before treatment (mmol/litre) | Glucose level 20 mins after treatment(mmol/litre) |
|---|---|---|
| *Psidium guajava* (small) | 4.7 | 3.3 |
| *Psidium guajava* (white) | 4.7 | 2.8 |
| *Psidium guajava* (pink) | 4.7 | 4.6 |
| *Psidium guajava* (hybrid) | 4.7 | 4.5 |
| *Syzygum cordatum* | 4.7 | 4.6 |
| *Eugenia jambulana* | - | - |
| *Clerodendrum L* | 5.0 | 4.0 |
| *Plantago major* | 5.0 | 5.1 |
| *Plantago lanceolata* | 3.2 | 3.5 |
| *Terminalia sericea* | 4.6 | 3.6 |
| *Prunus africana* | 4.6 | 4.9 |
| *Combretum molle* | - | - |
| *Buddleja salvifolia* | 4.6 | 5.2 |
| *Centella asiatica* | - | - |
| *Tetradenia riparia* | 4.1 | 3.4 |
| *Psychotreia serpeus* | - | - |

**All phytochemical extracts were injected intraperitoneally in a dose 40 mg/kg b.w.**
**- data not available**

## 4.7 CARDIOTONIC ACTIVITY OF TEST COMPOUNDS

### 4.7.1 Cardiotonic effects of the phytochemicals and pure triterpenoids in guinea pig atria

Table 12 and figures 30a-30d show the results of the cardiotonic effects of the phytochemicals and the isolated triterpenoids on reserpinised guinea pig atria. Of all the test compounds, *Plantago lanceolata, Prunus africana,* Oleanolic acid, Ursolic acid and uvaol displayed a positive inotropic effect on the electrically stimulated left atria. The

negative inotropic effects were displayed by methyl maslinate, *Syzygum cordatum, Combretum molle, Terminalia sericea,* all *Psidium guajava* subspecies, *and Eugenia jambolana.* On the other hand, *Clerodendrum capense, Plantago major, Psychotreia serpens, Buddleja salviifolia, Centella asiatica* and *Tetradenia riparia* displayed a dose-dependent biphasic effect. No phytochemicals had any effect on the chronotropic activity of the right atria.

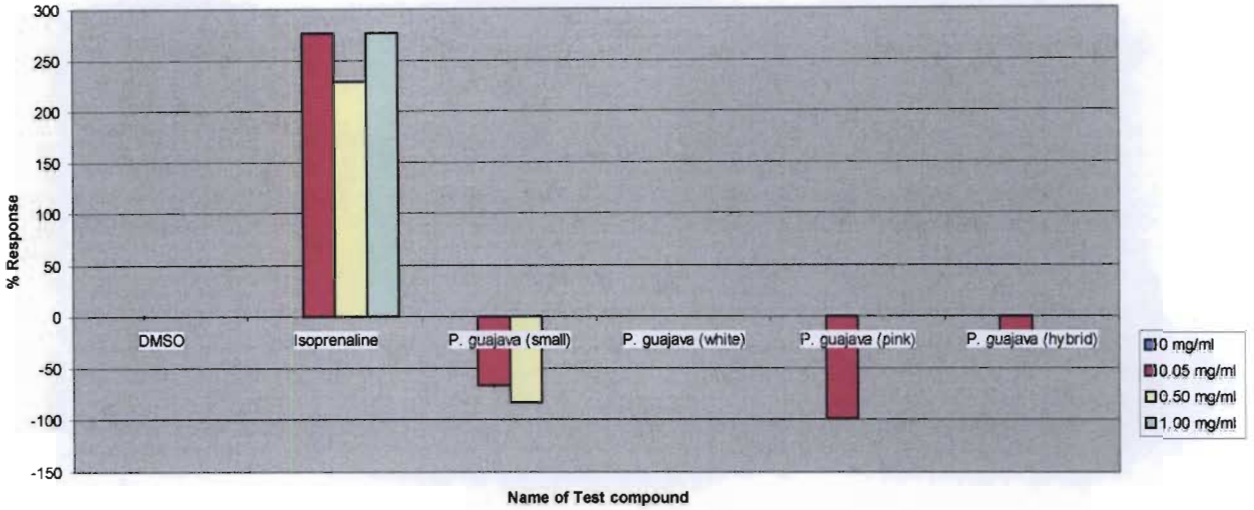


**Figure 30a. Dose-response relationship for the effect of crude ethyl acetate extracts on force of contraction as measured in electrically driven left guinea pig atria (means SEM; n= 3)**

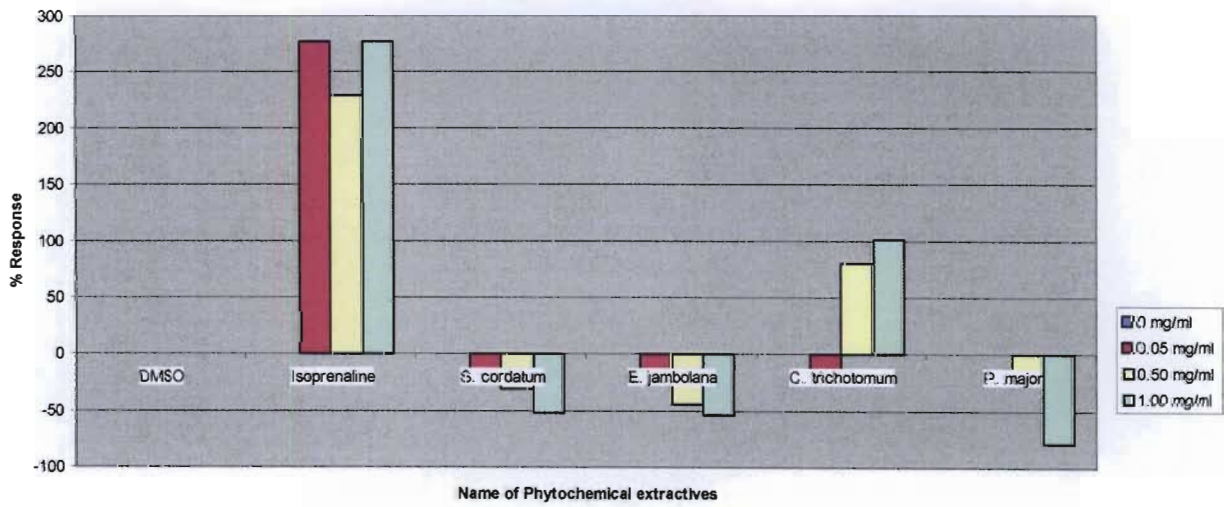


**Figure 30b. Dose-response relationship for the effect of phytochemical extractives on the force of contraction as measured in electrically driven left guinea pig atria (means SEM; n = 3)**

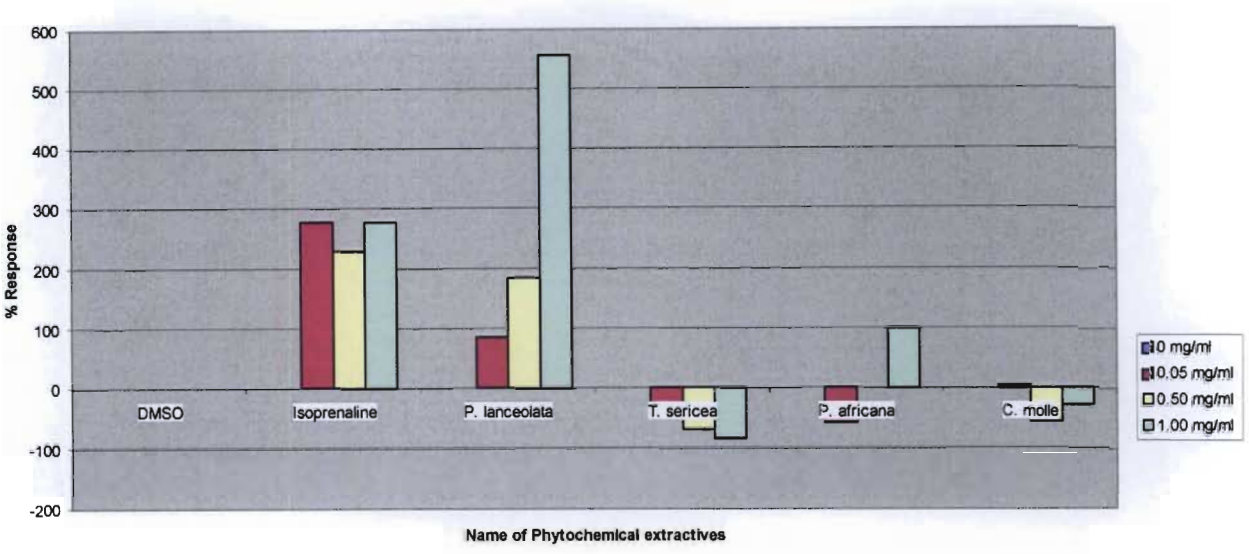


**Figure 30c. Dose-response relationship for the effect of phytochemicals on force of contraction as measured in electrically driven guineapig atria (means SEM; n = 3)**

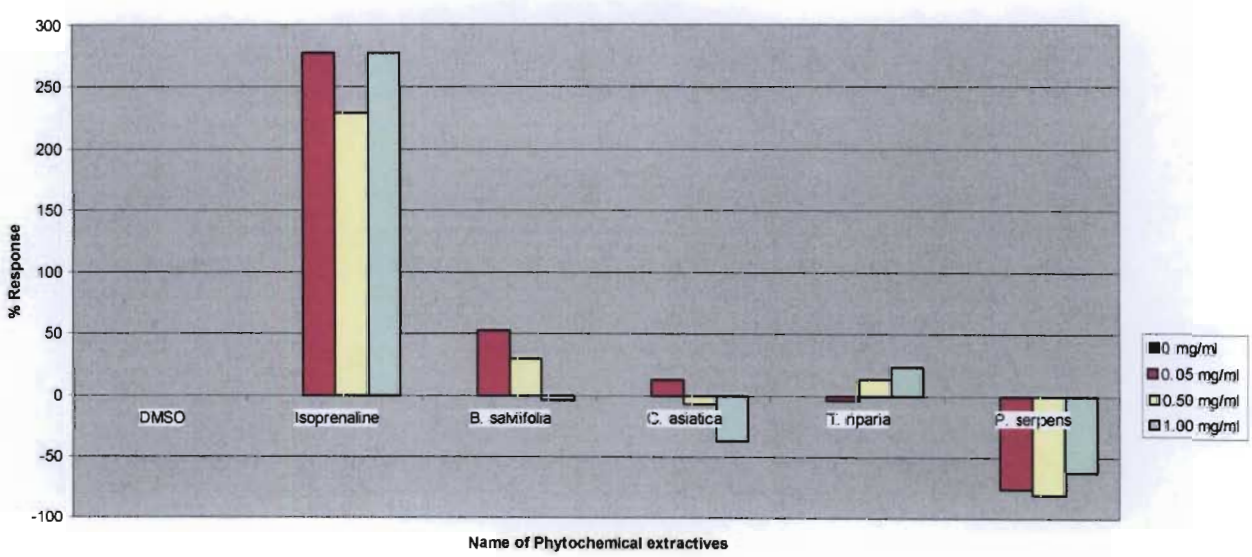


**Figure 30d. Dose-response relationship for the effect of the phytochemicals on force of contraction as measured in electrically driven left guinea pig atria (means SEM; n = 3)**

The pre-treatment of the atria with the oleanolic acid, ursolic acid and uvaol seem to potentiate the positive inotropic effect of isoprenaline, whilst methyl maslinate blocked the positive inotropic effect of isoprenaline (Figure 31). The effects of oleanolic acid, ursolic acid and uvaol was reversible as washing of the tissue reverted inotropic effect to their baseline values. On the other hand, the effects of methyl maslinate were not reversible

Pretreating the atria with propranolol completely blocked the inotropic effects of all the phytochemicals and the triterpenoids (Figure 32). Atropine sulphate had no effect on the inotropic effects of the triterpenoids.

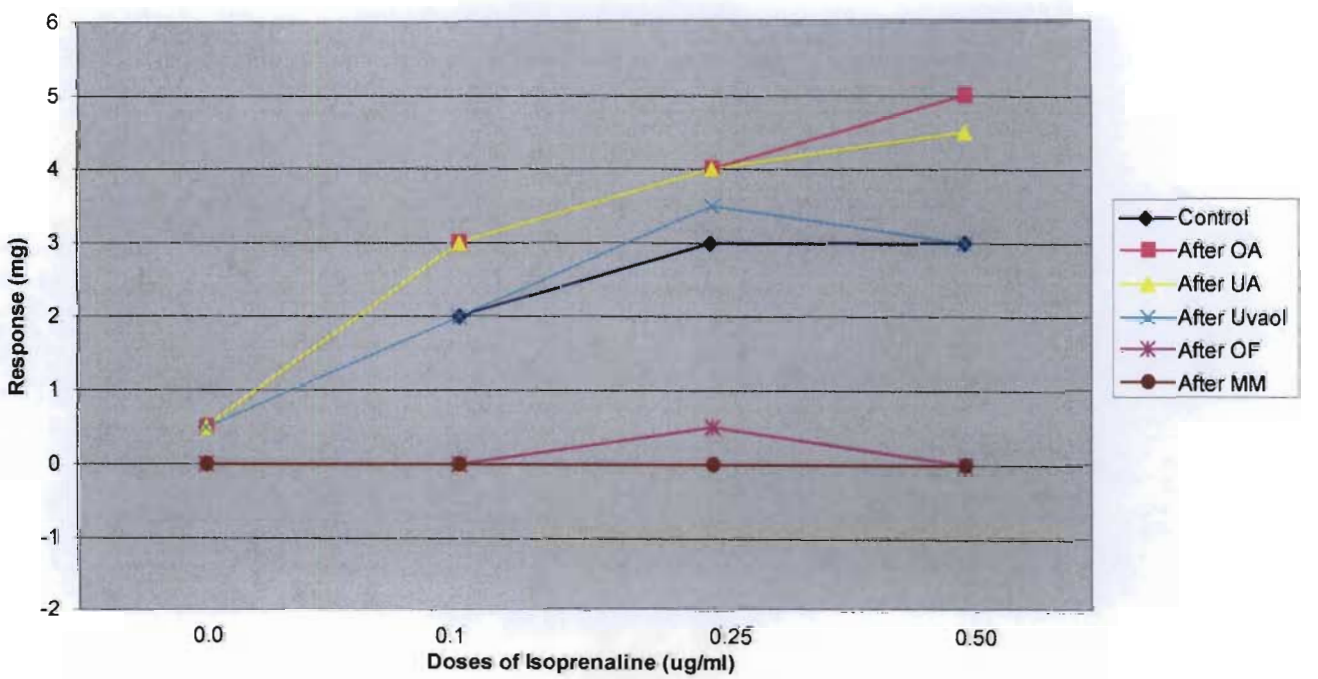


Figure 31. Effect of different doses of isoprenaline (ug/ml) on the background of different triterpenoids (1mg/ml) in electrically stimulated left guinea pig atria.

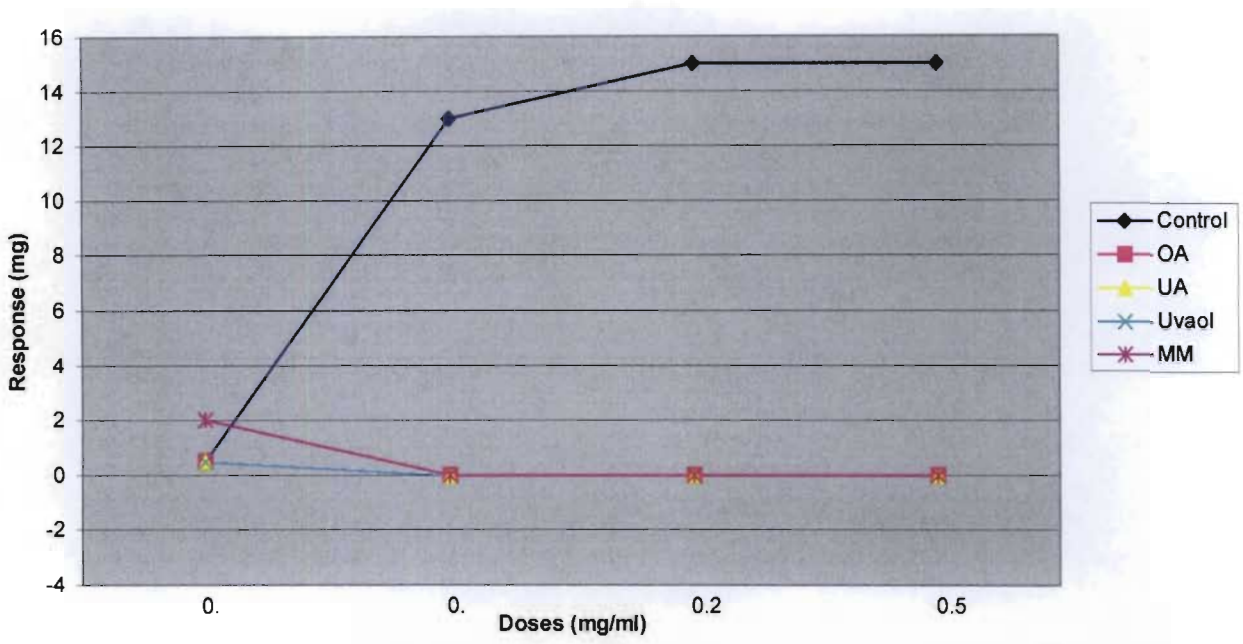


Figure 32. Effect of different doses of triterpenoids (mg/ml) on the background of propranolol (0.3mg/ml)

**Table 12: Evaluation of inotropic effects of test compounds on guinea pig atria**

| Parameter | Inotropic response (mg) of atria at different concentrations of test compounds (mg/ml) | | | |
|---|---|---|---|---|
| | **Baseline** | **0.05** | **0.50** | **1.00** |
| Isoprenaline | 2.83± | 10.67± ↑ 277 % | 9.33± ↑ 229 % | 10.67± ↑ 277 % |
| DMSO | 24± 0 | 24±0.00 | 24.0±0.000 | 24.0±0.000 |
| *Psidium guajava* (small fruit) | 3± 0 | 1.00±1.000 ↓ -67 % | 0.50±0.500 ↓ - 83 % | 0.00±0.000 ↓ |
| *Psidium guajava* (white fruit) | 2.25±1.750 | 0.00±0.00 ↓ | 0.00±0.000 | 0.00±0.000 |
| *Psidium guajava* (pink fruit) | 1.33±0.833 | 1.00±1.00 ↓ - 99 % | 0.00±0.000 | 0.00±0.000 |
| *Psidium guajava* (hybrid) | 5±0 | 4.00±0.00 ↓ -20 % | 0.00±0.000 | 0.00±0.000 |
| *Syzygum cordatum* | 4.83±4.086 | 3.50±2.784 ↓ - 27 % | 3.33±2.404 ↓ - 31 % | 2.33±1.364 ↓ - 52 % |
| *Eugenia jambulana* | 6.5±5.268 | 5.67±5.167 ↓ - 13 % | 3.67±3.667 ↓ - 44 % | 3.00±3.000 ↓ - 54 % |
| *Clerodendrum L* | 0.83±0.167 | 0.67±0.167 ↓ - 19 % | 1.50±0.500 ↑ 80 % | 1.67±1.202 ↑ 101 % |
| *Plantago major* | 2.33±1.856 | 2.33±1.856 | 1.83±1.590 ↓ - 21 % | 0.50±0.289 ↓ - 79 % |
| *Plantago lanceolata* | 1.17±0.441 | 2.17±1.424 ↑ 85 % | 3.33±2.833 ↑ 185 % | 6.50±3.329 ↑ 556 % |
| *Terminalia sericea* | 4.83±3.609 | 2.67±1.691 ↓ - 45 % | 1.50±0.764 ↓ - 69 % | 0.83±0.167 ↓ - 83 % |
| *Prunus africana* | 1.17±0.441 | 0.50±0.289 ↓ - 57 % | 1.17±0.601 | 2.33±0.667 ↑ 99 % |
| *Combretum molle* | 3.0±2.500 | 4.50±3.500 ↑ 50 % | 1.33±0.667 ↓ - 56 % | 2.17±1.922 ↓ - 28 % |
| *Buddleja salvifolia* | 5.0±4.500 | 7.67±7.167 ↑ 53 % | 6.50±6.252 ↑ 30 % | 4.83±4.586 ↓ - 3 % |
| *Centella asiatica* | 5.33±3.383 | 6.00±5.000 ↑ 13 % | 5.00±4.509 ↓ - 6 % | 3.33±1.667 ↓ - 37 % |
| *Tetradenia riparia* | 4.83±3.601 | 4.67±4.177 ↓ - 3 % | 4.17±3.919 ↓ - 14 % | 6.00±3.000 ↑ 24 % |
| *Psychotreia serpeus* | 3.5±1.607 | 0.83±0.601 ↓ - 76 % | 0.67±0.667 ↓ - 81 % | 1.33±0.882 ↓ - 62 % |

**Mean ± SEM** ↑ Positive inotropic effect ↓ Negative inotropic effect

*** The difference is significant compared to the control value**

### 4.7.2 Cardiotonic effects of the pure triterpenoids in anaesthetised wistar rats

The inotropic and dromotropic effects of the triterpenoids in anaesthetised rats are presented in table 13. All the triterpenoids had a positive inotropic effect on the rats as shown by the increase in the amplitude of the QRS complex. The highest increases in the inotropic activity were shown by oleanolic acid and uvaol. It is interesting to note that even methyl maslinate (MM) had a positive inotropic effect in the rat model unlike in the guinea pig model where it had a negative inotropic effect. In terms of the dromotropic effects, only oleanolic acid increased the PQ interval.

## 4.8 ANTIARRHYTHMIC ACTION OF TEST COMPOUNDS

### 4.8.1 On $CaCI_2$- induced arrhythmia

*Psidium guajava* (white fruit), *Syzygum cordatum, Buddleja salviifolia*, oleanolic acid and ursolic acid displayed antiarrhythmic effects on $CaCl_2$-induced arrhythmia. These antiarrhythmic effects were comparable to those of amiodarone. *Centella asiatica, Tetradenia riparia* and *Psychotreia serpens* displayed an antiarrhythmic activity that was comparable to that of verapamil (table 14).

### 4.8.2 On adrenaline-induced arrhythmia

*Psidium guajava* (hybrid), *Eugenia jambolana, Prunus africana, Terminalia riparia*, oleanolic acid and ursolic conferred an antiarrhythmic effect on the adrenaline-induced

arrhythmia (Table 15). This protective effect was comparable to that of conferred by propranolol.

### 4.8.3 On $BaCl_2$-induced arrhythmia

Table 16 shows the result of the antiarrhythmic effects of the phytochemicals and the triterpenoids on $BaCl_2$-induced arrhythmia. *Tetradenia riparia* displayed an antiarrhythmic effect on this model that was comparable to that of propranolol. Ursolic acid and oleanolic acid displayed antiarrhythmic effects that were similar to those of amiodarone.

### 4.8.4 On Ischemia-reperfusion arrhythmia

Ursolic acid and methyl maslinate displayed antiarrhythmic activity on ischaemic-reperfusion induced arrhythmia that were comparable to that of verapamil (Table 17). On the other hand uvaol and oleanolic acid displayed antiarrhythmic effects that were similar to that of propranolol.

**Table 13: Evaluation of dromotropic (PQ interval) and inotropic (QRS complex) effects of triterpenoids isolated from plants**

| | Baseline | | 3 minute | | 5 minute | | 10 minute | | 20 minute | | 30 minute | | 40 minute | | 60 minute | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Treatment** | **PQ (sec)** | **QRS (mV)** | **PQ (sec)** | **QRS (mV)** | **PQ (sec)** | **QRS (mV)** | **PQ (sec)** | **QRS (mV)** | **PQ (sec)** | **QRS (mV)** | **PQ (sec)** | **QRS (mV)** | **PQ (sec)** | **QRS (mV)** | **PQ (sec)** | **QRS (mV)** |
| Oleanolic acid i.p. (40 mg/kg) | 0.020 ± 0.002 | 0.141 ± 0.006 | 0.025 ± 0.002 | 0.162 ± 0.006 * ↑ 15% | 0.040 ± 0.001 * ↑ x 2 | 0.166 ± 0.006 * ↑ 18% | 0.040 ± 0.001 * ↑ x 2 | 0.181 ± 0.002 * ↑ 28% | 0.040 ± 0.001 * ↑ x 2 | 0.188 ± 0.005 * ↑ 33% | 0.040 ± 0.001 * ↑ x 2 | 0.207 ± 0.003 * ↑ 46% | 0.040 ± 0.001 * ↑ x 2 | 0.192 ± 0.001 * ↑ 36% | 0.040 ± 0.002 * ↑ x 2 | 0.201 ± 0.001 * ↑ 43% |
| Ursolic acid i.p (40 mg/kg) | 0.028 ± 0.002 | 0.268 ± 0.002 | 0.027 ± 0.002 | 0.283 ± 0.006 ↑ 5% | 0.020 ± 0.001 | 0.287 ± 0.003 * ↑ 7% | 0.023 ± 0.002 | 0.294 ± 0.004 * ↑ 10% | 0.022 ± 0.002 | 0.296 ± 0.003 * ↑ 10% | 0.025 ± 0.002 | 0.293 ± 0.003 * ↑ 9% | 0.023 ± 0.003 | 0.298 ± 0.006 * ↑ 11% | 0.022 ± 0.002 | 0.296 ± 0.003 * ↑ 10% |
| Methyl maslinate acid i.p. (40 mg/kg) | 0.018 ± 0.002 | 0.120 ± 0.007 | 0.020 ± 0.002 | 0.143 ± 0.006 * ↑ 19% | 0.015 ± 0.002 | 0.153 ± 0.006 * ↑ 28% | 0.020 ± 0.001 | 0.180 ± 0.007 * ↑ 50% | 0.025 ± 0.003 ↑ 38% | 0.170 ± 0.007 * ↑ 42% | 0.025 ± 0.003 ↑ 38% | 0.184 ± 0.006 * ↑ 53% | 0.020 ± 0.001 | 0.180 ± 0.005 * ↑ 50% | 0.020 ± 0.002 | 0.170 ± 0.006 * ↑ 42% |
| Uvaol i.p. (40 mg/kg) | 0.015 ± 0.003 | 0.120 ± 0.007 | 0.010 ± 0.002 | 0.150 ± 0.006 * ↑ 25% | 0.015 ± 0.002 | 0.246 ± 0.006 * ↑ x 2 | 0.015 ± 0.001 | 0.229 ± 0.007 * ↑ x 2 | 0.015 ± 0.002 | 0.245 ± 0.006 * ↑ x 2 | 0.015 ± 0.002 | 0.182 ± 0.006 * ↑ 52% | 0.018 ± 0.002 | 0.180 ± 0.006 * ↑ 50% | 0.015 ± 0.003 | 0.186 ± 0.007 * ↑ 55% |

**Mean** ± SEM

* **The difference is significant compared to control values**

**i.p. Intraperitoneally**

**TABLE 14: Effects of antidysrhythmic drugs and phytochemical extracts on $CaCl_2$ – induced arrhythmia**

| | Control | | | $CaCl_2$ (140 mg/kg) intravenously | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Heart rate decrease (%) | | | Heart rate decrease (%) | | | | | |
| **Treatment** | **1 min** | **10 min** | **20 min** | **1 min** | **3 min** | **VPB (%)** | **VF (%)** | **Restoration of sinus rhythm (sec)** | **Mortality (%)** |
| Saline | 0 | 0 | 0 | 20 | 26 | 100 | 80 | 40 ± 5 | 67 |
| Quinidine p.o. (5 mg/kg) | 5 | 12 | 9 | 8 | 6 | 100 | 80 | 60 ± 8 | 60 |
| Propranolol i.v (2 mg/kg) | 25 | 30 | 30 | 20 | 20 | 100 | 60 | 60 ± 6 | 60 |
| Amiodarone p.o (10 mg/kg) | 4 | 4 | 2 | 14 | 6 | 80 | 12 | 10 ± 3 | 10 |
| Verapamil i.v. (2 mg/kg) | 18 | 30 | 32 | 10 | 8 | 20 | 0 | 20 ± 2 | 4 |
| Oleanolic acid i.p. (10 mg/kg) | 0 | 4 | 12 | 1 | 1 | 60 | 8 | 10 ± 3 | 16 |
| Ursolic acid i.p (10 mg/kg) | 1 | 10 | 18 | 1 | 1 | 60 | 8 | 10 ± 4 | 18 |
| *P. guajava* (small) | 0 | 4 | 6 | 7 | 7 | 80 | 10 | 60 ± 3 | 30 |
| *P. guajava* (white) | 0 | 6 | 0 | 6 | 6 | 60 | 8 | 30 ± 4 | 20 |
| *P. guajava* (pink) | 0 | 9 | 6 | 3 | 3 | 80 | 12 | 60 ± 4 | 30 |
| *P. guajava* (hybrid) | 0 | 12 | 6 | 7 | 0 | 80 | 20 | 60 ± 4 | 40 |
| *Syzygum cordatum* | 0 | 0 | 13 | 7 | 0 | 60 | 20 | 60 ± 3 | 40 |
| *Eugenia jambulana* | 0 | 20 | 22 | 20 | 20 | 80 | 80 | 0 | 68 |
| *Clerodendrum L* | 0 | 10 | 14 | 13 | 7 | 80 | 80 | 60 ± 4 | 60 |
| *Plantago major* | 0 | 6 | 6 | 12 | 12 | 80 | 80 | 60 ± 2 | 60 |
| *Plantago lanceolata* | 0 | 0 | 0 | 10 | 0 | 100 | 80 | 0 | 80 |
| *Terminalia sericea* | 0 | 0 | 0 | 7 | 0 | 80 | 80 | 40 ± 4 | 60 |
| *Prunus africana* | 0 | 6 | 6 | 7 | 7 | 80 | 80 | 60 ± 4 | 60 |
| *Combretum molle* | 0 | 13 | 13 | 13 | 10 | 80 | 80 | 60 ± 4 | 60 |
| *Buddleja salvifolia* | 0 | 18 | 20 | 0 | 0 | 60 | 60 | 60 ± 2 | 60 |
| *Centella asiatica* | 0 | 7 | 7 | 0 | 0 | 40 | 40 | 30 ± 6 | 40 |
| *Tetradenia riparia* | 0 | 7 | 8 | 0 | 0 | 40 | 40 | 40 ± 6 | 30 |
| *Psychotreia serpeus* | 0 | 7 | 7 | 0 | 0 | 40 | 20 | 30 ± 4 | 10 |

**All phytochemical extracts were injected intraperitoneally in a dose 100 mg/kg b.w.**
**Sinus rhythm restoration of the survived rats: Mean ± SEM**
**VPB – Ventricular premature beats VF - Ventricular fibrillation**
**p.o. - Orally i.v. – Intravenously i.p. - Intraperitoneally**

**Table 15: Effects of antidysrhythmic drugs and phytochemical extracts on adrenaline-induced arrhythmia**

| | Control | | | Adrenaline (30 µg/kg) intravenously | | | | |
|---|---|---|---|---|---|---|---|---|
| | Heart rate decrease (%) | | | | | | | |
| **Treatment** | **1 min** | **10 min** | **20 min** | **VPB (%)** | **VT (%)** | **VF (%)** | **Restoration of sinus rhythm (sec)** | **Mortality (%)** |
| Saline | 0 | 0 | 0 | 100 | 100 | 100 | 0 | 66 |
| Quinidine p.o. (5 mg/kg) | 4 | 4 | 8 | 80 | 60 | 60 | 60 ± 11 | 60 |
| Propranolol i.v (2 mg/kg) | 20 | 15 | 15 | 20 | 0 | 0 | 20 ± 8 | 0 |
| Amiodarone p.o (10 mg/kg) | 2 | 9 | 2 | 100 | 80 | 80 | 0 | 60 |
| Verapamil i.v. (2 mg/kg) | 30 | 25 | 20 | 80 | 80 | 60 | 0 | 40 |
| Oleanolic acid i.p. (10 mg/kg) | 6 | 21 | 21 | 20 | 2 | 8 | 10 ± 6 | 8 |
| Ursolic acid i.p (10 mg/kg) | 3 | 5 | 5 | 40 | 10 | 8 | 14 ± 7 | 10 |
| *P. guajava* (small) | 0 | 20 | 20 | 80 | 20 | 50 | 120 ± 10 | 30 |
| *P. guajava* (white) | 0 | 20 | 20 | 80 | 10 | 50 | 120 ± 8 | 30 |
| *P. guajava* (pink) | 0 | 20 | 20 | 100 | 0 | 60 | 80 ± 8 | 30 |
| *P. guajava* (hybrid) | 0 | - | 7 | 20 | 0 | 20 | 30 ± 6 | 20 |
| *Syzygum cordatum* | 0 | 14 | 14 | 100 | 20 | 50 | 60 ± 10 | 40 |
| *Eugenia jambulana* | 0 | 9 | 6 | 50 | 0 | 20 | 30 ± 8 | 20 |
| *Clerodendrum L* | 0 | 6 | 6 | 80 | 2 | 20 | 30 ± 6 | 40 |
| *Plantago major* | 0 | 5 | 5 | 80 | 40 | 20 | 30 ± 8 | 40 |
| *Plantago lanceolata* | 0 | 8 | 10 | 50 | 20 | 10 | 30 ± 4 | 40 |
| *Terminalia sericea* | 0 | 20 | 20 | 80 | 0 | 60 | 30 ± 6 | 30 |
| *Prunus africana* | 0 | 15 | 18 | 50 | 0 | 0 | 45 ± 4 | 40 |
| *Combretum molle* | 0 | 6 | 3 | 50 | 50 | 20 | 30 ± 4 | 40 |
| *Buddleja salvifolia* | 0 | 20 | 20 | 60 | 0 | 60 | 60 ± 8 | 40 |
| *Centella asiatica* | 0 | 3 | 0 | 50 | 50 | 20 | 30 ± 6 | 40 |
| *Tetradenia riparia* | 0 | 18 | 13 | 50 | 0 | 0 | 45 ± 6 | 20 |
| *Psychotreia serpeus* | 0 | 6 | 12 | 50 | 0 | 20 | 30 ± 4 | 20 |

**All phytochemical extracts were injected intraperitoneally in a dose 100 mg/kg b.w.**
**Sinus rhythm restoration of the survived rats: Mean ± SEM**
**VPB – Ventricular premature beats** **VT - Ventricular tachycardia**
**VF - Ventricular fibrillation** **p.o. - Orally**
**i.v. – Intravenously** **i.p. - Intraperitoneally**

**Table 17: Effects of antidysrhythmic drugs and triterpenoids on Ischemia-reperfusion induced arrhythmia in anaesthetised rats**

| | 30 minute myocardial ischaemia | | | 20 minute reperfusion | | | |
|---|---|---|---|---|---|---|---|
| **Treatment** | **VPB (%)** | **VT (%)** | **VF (%)** | **VPB (%)** | **VT (%)** | **VF (%)** | **Total mortality %)** |
| Saline-treated preconditioned | 77 | 77 | 22 | 22 | 33 | 0 | 22 |
| Saline-treated non-preconditioned | 89 | 77 | 67 | 44 | 44 | 44 | 67 |
| Quinidine p.o. (5 mg/kg) | 83 | 66 | 17 | 33 | 33 | 0 | 17 |
| Propranolol i.v (2 mg/kg) | 66 | 33 | 17 | 33 | 33 | 33 | 33 |
| Amiodarone p.o (10 mg/kg) | 66 | 66 | 17 | 33 | 33 | 0 | 17 |
| Verapamil i.v. (2 mg/kg) | 83 | 66 | 50 | 66 | 50 | 50 | 50 |
| Oleanolic acid i.p. (40 mg/kg) | 66 | 33 | 33 | 33 | 33 | 33 | 33 |
| Ursolic acid i.p (40 mg/kg) | 83 | 50 | 50 | 66 | 50 | 50 | 50 |
| Methyl maslinate acid i.p. (40 mg/kg) | 83 | 66 | 66 | 83 | 50 | 66 | 66 |
| Uvaol i.p. (40 mg/kg) | 66 | 33 | 33 | 33 | 33 | 33 | 33 |

**Each group consisted of six rats except the two control (saline) groups that consisted of nine rats each**

**VPB – Ventricular premature beats**

**VT - Ventricular tachycardia**

**VF - Ventricular fibrillation**

**p.o. - Orally**

**i.v. - Intravenously**

**i.p. – Intraperitoneally**

## 4.9- CONFIRMATION OF OCCURRENCE OF ISCHAEMIA

Gross examination of the hearts under ultraviolet light confirmed that ligation of the coronary artery did cause ischaemia to occur. Figure 33 shows an example of a heart that displays blue areas indicating presence of non-perfused areas of a myocardium. On the other hand, figure 34 shows greenish areas that is an indication of the presence of perfused areas.

Figure 33 Representative heart in which ischaemia had occurred

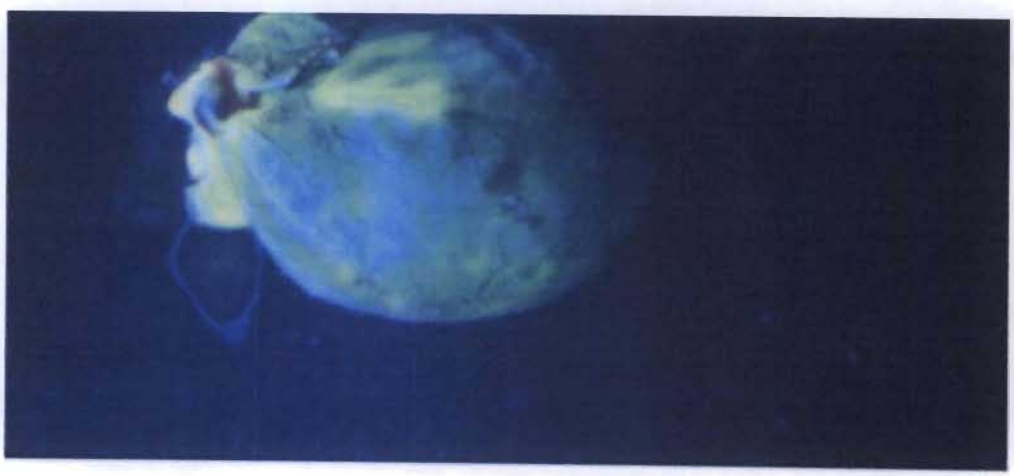

Figure 34. Representative heart in which ischaemia had not occurred

# 5.0 DISCUSSION

## 5. INTRODUCTION

The working hypothesis of this study was that triterpenoids of plant origin might provide cheap and accessible traditional medicine source for treatment of complicated hypertension if they have cardiotonic and antiarrhythmic effects in addition to their hypotensive effect. In order to test this hypothesis, pharmacological analysis of 16 different phytochemicals and 4 triterpenoids isolated from these phytochemicals was done. The analysis revealed that the triterpenoids and all the phytochemicals had determined physiological activity. As basic features, the isolated triterpenoids and some of the crude extracts had hypotensive, cardiotonic and antiarrhythmic effects on different models of hypertension as is shown in the summary of results below. Thus, the study demonstrated that triterpenoids of plant origin might provide cheap and accessible traditional medicine source for treatment of complicated hypertension.

### 5.1 Summary of Results

- The study reconfirmed the presence of triterpenoids in the selected medicinal plants
- All the crude extracts and the isolated triterpenoids had a low toxicity
- All the test compounds had acute and long-lasting vasodepressor activity in rats
- The test compounds displayed natriuretic and diuretic effects that were comparable and sometimes better than those displayed by hydrochlorothiazide

- OA, UA, UV, *Plantago lanceolata* and *Prunus africana* displayed a positive inotropic effect on guinea pig atria. This inotropic effect was blocked by propranolol
- *Plantago major, Psychotria serpens, Buddleja salviifolia, Centella asiatica, Tetradenia riparia* and *Clerodendrum trichotomum* displayed a dose-dependant biphasic inotropic effect.
- *Syzygium cordatum, Eugenia jambolana, Combretum molle, Terminalia sericea*, and all the four *Psidium guajava* subspecies displayed a negative inotropic effect.
- All the isolated triterpenoids conferred antiarrhythmic effects on both chemically induced and ischaemia-reperfusion induced arrhythmia.
- *Psidium guajava* (hybrid), *Eugenia jambolana, Prunus africana* and *Terminalia riparia* displayed an antiarrhythmic effect against adrenaline-induced arrhythmia whilst *Psidium guajava* (white fruit), *Syzygium cordatum* and *Buddleja salviifolia* displayed antiarrhythmic effects against $CaCl_2$ induced arrhythmia.
- Crude extracts from *Psidium guajava* (small fruit) *Psidium guajava* (white fruit), *Clerodendrum capense, Terminalia sericea* and *Tetradenia riparia* displayed a potent hypoglycaemic activity.

The specific biological profiles of the triterpenoids and the phytochemicals that have been investigated in this study will be discussed below.

**5.2 Isolation of crude and pure compounds**

The study has reconfirmed that many medicinal plants contain triterpenoids as part of their chemical constituents. In particular, the study has shown that all the medicinal plants that

were screened in the study have a certain percentage of triterpenoids in agreement to what was reported in the Anne Hutchings' inventory of Zulu Medicinal Plants (1996). The plants that were screened were *Psidium guajava* (small fruit), *Psidium guajava* (white fruit), *Psidium guajava* subsp. *durbanse* (hybrid), *Psidium guajava* (pink fruit), *Plantago major, Psychotria serpeus, Plantago lanceolata, Clerodendrum trichotomum, Eugenia jambulana, Buddleja salviifolia., Combretum molle, Centella asiatica, Tetradenia riparia, Syzygium cordatum, Prunus africana and Terminalia sericea.* The study was able to isolate four different triterpenoids namely, oleanolic acid (OA), ursolic acid (UA), uvaol (UV) and methyl maslinate (MM). These triterpenoids were isolated from the crude ethyl acetate extracts.

The most abundant triterpenoids was OA followed by UA then UV and lastly MM. OA recrystallised from methanol as colourless crystals with a melting point of about 307-308 $^{o}$C. Its spectral data were identical with literature values (Maillard *et al.*, 1992). UA recrystallised from methanol as colourless crystals with melting point of 286 $^{0}$C. Its spectral data were identical with literature values (Seo *et al.*, 1975a). UV recrystallised from methanol/dichloromethane as colourless crystals with a melting point of about 221-223 $^{o}$C. Its spectral data were identical with literature values (Siddiqui *et al.*, 1986). MM was only eluted when the crude ethyl acetate extract was subjected to a silica gel column chromatography with gradient elution using a 60 %/ 40 % hexane/ethyl acetate solvent system. The eluates containing MM were obtained only from the last fractions. MM was used without recrystallisation, and its spectral properties were identical with literature values (Seo *et al.*, 1975b)

It should be noted that the calculated percentage yield is by no means the total value found in the plants. It is very likely that some of the triterpenoids were left in the methanol, hexane and dichloromethane crude extracts. This fact is backed by the results from other studies that have shown that the triterpenoids were extracted using the above solvents. For instance, there are reports that showed that ursolic acid was found in the methanol crude extract of *Psychotria serpens* (Lee, *et al.*, 1988) and in the hexane crude extract of *Plantago major* (Ringbom, *et al.*, 1998). Thus, it is safe to assume that the estimated percentage yield of triterpenoids presented in this study is lower than the actual value as it was calculated only from the crude ethyl acetate extracts. Nevertheless, of the crude ethyl acetate extracts the highest yield was obtained from *Buddleja salvifolia* and *Syzygium cordatum* whilst the least yield was from *Tetradenia riparia* and *Centella asiatica.*

All the crude ethyl acetate extracts and the isolated triterpenoids showed low toxicity when tested for toxicity using the brine shrimp test. The Hippocratic test on the animals to qualitatively determine toxicity was not done in this study due to cost constraints. However, previous studies have shown that in a 5 day follow up study, Hippocratic test of OA and UA on rats had low toxicity (Somova, *et al.*, 2003a and 2003b). Thus the therapeutic values of the triterpenoids would not be contraindicated by the toxic effect as is the case with other plant derivatives like glycosides.

### 5.3 Hypotensive effects of the phytochemicals and the triterpenoids

The study has shown that the isolated triterpenoids and most of the phytochemicals had a hypotensive effect in Wistar rats. All the four triterpenoids showed a significant vasodepressor effect and sinus bradycardia that lasted for more than 60 min. These effects

were dose-dependent with the peak effect seen at 20 min after application. The most potent effects were shown by OA and MM. This finding is in disagreement with the results presented by Kolah *et al* (2001) who showed that ursolic acid, oleanolic acid and α-amyrin had no vasodepressor effect in anaesthetised male Wistar rats. However, our results are in agreement with other studies that have also showed that triterpenoids might have hypotensive effects (Somova, *et al.*, 2003a and 2003b).

As has been stated in the previous sections, mean systemic arterial pressure (MAP) is the product of cardiac output (CO) and the total peripheral resistance (TPR) that is the sum of all resistances to flow offered by all the systemic blood vessels. Thus, MAP = CO x TPR. Cardiac output is affected by stroke volume and heart rate whilst total peripheral resistance is mainly affected by the tone of the arterioles, which are the resistance vessels. There is a strong causal, reciprocal relationship between arterial blood pressure and blood volume, and as a result, blood volume is the major long-term determinant of blood pressure (Vander, 2001). On the other hand, baroreceptor reflexes that cause a decrease in the sympathetic firing and in the total peripheral resistance induce the immediate changes in blood pressure.

In this study, the test compounds caused blood pressure decrease within 10 min and the hypotensive effect was sustained for a long time. This observation was suggestive of the involvement of short-term blood pressure regulating mechanism and a long-term blood pressure regulating mechanism. The short-term blood pressure regulating mechanism can be postulated to involve receptors that cause a decrease in total peripheral resistance. This point is strengthened by the observation that some test compounds displayed a positive inotropic effect. The presence of positive inotropic activity and a hypotensive activity is a

contradiction since one would expect the positive inotropic activity to cause an increase in the cardiac output that will in turn lead to an increase in blood pressure. Involvement of the reduction in total peripheral resistance is the probable way in which the effect of the increased cardiac output was offset. We postulate that that the test compounds might have caused a reduction in the total peripheral resistance that resulted in a decrease in the mean arterial pressure. The test compounds might have reduced the total peripheral resistance by either having an antagonistic effect on the vascular α-adrenergic receptors which when stimulated cause vasoconstriction. The other possibility might have involved the stimulation of the endothelial cells and/or the noncholinergic, nonadrenergic neurons to release vasodilators like nitric oxide. The vasodilators in turn act on adjacent vascular smooth muscles to cause vasodilatation. Thus, the postulated vasodilatation and the blocking of α-adrenergic receptors might have a caused a reduction in the total peripheral resistance. Since, MAP = CO x TPR, the postulated reduction in TPR should have caused the observed immediate hypotensive effect of the test compounds.

The long-lasting hypotensive effect can be partly attributed to the diuretic and natriuretic effects that were displayed by these test compounds. These effects were shown to be long lasting since they lasted for at least 24 hours. The high $Na^+/K^+$ ratio of the treated animals was indicative of a reduced sodium retention in the loop of Henle. Thus, in terms of the pressure natriuresis curve, it is most probably shifted to the left whereby at any given pressure the kidneys are excreting more $Na^+$. There might also be the possibility of an increased glomerular filtration rate that might contribute to the increased natriuresis. The increased natriuresis and diuresis caused by the isolated triterpenoids and the crude extracts might have caused a decrease in plasma volume that sequentially decreased blood volume, venous pressure and finally venous return. In accordance with the Frank-Starling

mechanism, the decreased venous return caused a decrease in cardiac output by decreasing end-diastolic volume and hence stroke volume. In some cases, in addition to the reduction in stroke volume, there was a reduction in heart rate. The reduction in stroke volume and heart rate led to a decrease in cardiac output (CO). Since MAP = CO x TPR, the decrease in cardiac output led to the observed long-lasting reduction in blood pressure.

The hypotensive effect might also come about through the vasodilating effect caused by the binding of the triterpenoids on the β-adrenergic receptors of arteriolar smooth muscles of the skeletal muscles. Because arterioles in skeletal muscles have large numbers of β-adrenergic receptors, agonists of these receptors cause dilation in this vascular bed.

The above hypotensive effects suggest that the triterpenoids would be ideal for the treatment of hypertension in black hypertensive subjects. Black hypertensives have been reported to have a higher plasma sodium concentration than normotensives (Weissberg, *et al.*, 1987). Furthermore, it is generally accepted that thiazides diuretics are effective antihypertensive agents in black hypertensive patients and that they cause a greater decrease in blood pressure in blacks (VACSAA, 1982; Moser and Lunn, 1982; Chobanian *et al.*, 2003). This better hypotensive response in black patients is postulated to be because more blacks than white have an expanded intracellular volume and low plasma renin activity (Seedat, 2000). The thiazides diuretic are ideal for most developed countries due to their low cost. In the same vain, since our phytochemicals have shown to have diuretic and natriuretic effects comparable and in some cases similar to hydrochlorothiazide, it can be suggested that the phytochemicals would be ideal to be used as a treatment of hypertension in Africa. The accessibility and abundant availability of these phytochemicals in Africa would make them ideal as a treatment for hypertension

considering that the health care resources in this region are particularly scarce. Thus, the use of these triterpenoids for the treatment of hypertension would be proposed and encouraged since they share some similarities with thiazide diuretics.

Diuretics have also been reported to enhance the antihypertensive efficacy of multidrug regimens in addition to their being more affordable and achieving blood pressure control than other antihypertensive agents (Chobanian *et al.*, 2003). This fact solidifies the assertion that the triterpenoids and some phytochemicals that have been shown to have diuretic effects should be used as adjuvant drugs to the western medicine as part of a multidrug therapy.

**5.4 Cardiotonic effects of the phytochemicals and the triterpenoids**

The study has shown that the triterpenoids had a positive inotropic effect on both isolated guinea pig atria and in vivo in anaesthetised rats except methyl maslinate that did not cause a positive inotropic effect in the in vitro experiments. The most potent positive inotropic effects were displayed by oleanolic acid and uvaol. The onset of the inotropic action was quick and the action was long lasting in both models. In the in vivo experiments, the action lasted for more 60 minutes.

From the cardiotonic analysis, it was observed that though the inotropic response values varied a lot between tissues, the extracts caused a persistent trend in the inotropic actions. It was impossible to calculate the statistic differences between the baseline values and the dose response values due to the limited number of tissues tested per test compound. Nevertheless, the values obtained maintained a consistent trend that was enough to infer conclusions from.

Atropine sulphate, a cholinergic receptor inhibitor, did not block the inotropic effects of the triterpenoids. Thus, it can be postulated that the observed positive inotropic effects of our triterpenoids were not mediated by muscarinic receptors. We can also speculate that the positive inotropic effects were not mediated indirectly via liberation of catecholamines. This speculation is backed by the demonstration of the inotropic action in the reserpine-treated guinea pig atria. However, the positive inotropic effects of the triterpenoids were blocked by a β-adrenergic blocker, propranolol. This inhibition demonstrates that the positive inotropic effects of the triterpenoids were mediated through β-adrenergic receptors.

The experiments of the cardiotonic effect of isoprenaline after pre-treatment with the triterpenoids showed that OA, UV and UA had a potentiating effect on isoprenaline. The action was not additive since the effect of isoprenaline on the background of the triterpenoids was more than the maximum effect observed with isoprenaline alone. The study did not ascertain as through which mechanism the potentiating effect was done. We can only speculate on a number of possibilities that the potentiation might have come about. Firstly, the binding of triterpenoids to the β-receptors might have caused a conformational change (allosteric modifications) to the adenylate cyclase complex that enhances the activity of isoprenaline. This mechanism has been shown to happen in brain tissues whereby pre-treatment of the tissues with forskolin potentiated the effect of hormones (Seamon, *et al.*, 1984). The second possibility is that the triterpenoids in addition to their acting through the receptors might also be working downstream to the receptors. In this case, the triterpenoids might have cause an increase in the free $Ca^{2+}$ concentration. The increase would be either through the inhibition of phosphodiesterase or through an increase in the release of $Ca^{2+}$ from $Ca^{2+}$ stores like the sarcoplasmic reticulum.

The last possibility is that the triterpenoids might have increased the sensitivity of the contractile apparatus for $Ca^{2+}$. This mechanism has been shown to be displayed by the *n*-butanolic fraction from *Berberis aristata* fruit (Gilani *et al.,* 1999).

Of all the plants screened, only *Plantago lanceolata* and *Prunus africana* displayed a positive inotropic action. A Medline search demonstrated that this is the first time that a positive inotropic effect of these plants has been reported. This inotropic effect was also blocked by a β-adrenoceptor blocker, propranolol, indicating that the effect was mediated through the β-adrenergic receptors. What was more intriguing was that these plants did not yield the highest percentage of triterpenoids in comparison to the other screened plants. This seem to suggest that the positive inotropic action of the two plants might be due to a synergistic effect of the triterpenoids and some other yet to be identified compound. It also showed that, for therapeutic purposes, a crude extract of the whole plant leaves might have better effects.

A number of the plants screened displayed a dose-dependant biphasic inotropic effect. The plants that had this effect are *Plantago major, Psychotria serpens, Buddleja salviifolia, Centella asiatica, Tetradenia riparia* and *Clerodendrum trichotomum.* These plants had a positive inotropic effect at one concentration and a negative inotropic effect at another concentration. This is not the first time that this kind of action has been reported since other studies of plant extracts have also shown it. For instance, Pennacchio, *et al.*, (1995) showed that *Eremophila alternifolia* extracts displayed a biphasic inotropic effect. This biphasic was not mediated by neither α-adrenergic nor β-adrenergic receptors.

The study also showed that some of the screened plants have a negative inotropic effect on a guinea pig atria. The plants that had this action are *Syzygium cordatum, Eugenia jambolana, Combretum molle, Terminalia sericea*, and all the four *Psidium guajava* subspecies. The mechanism of action was not demonstrated in this study. However, in case of the guavas, the negative inotropic effect was probably mediated through the cholinergic receptors. Conde Garcia *et al.*, (2003) have recently shown that the negative inotropic effect of guavas is not blocked by propranolol nor naloxone but by atropine sulphate. Thus, the negative inotropic effect did not involve β-adrenergic receptors nor opiod membrane receptors but rather through cholinergic receptors. The authors assert that the guavas mostly probably inhibited $Ca^{2+}$ inward currents thereby causing a negative inotropic effect. These negative inotropic agents might have the potential of being used in the treatment of hypertrophic cardiomyopathy whereby they can improve cardiovascular hemodynamics. They also point to their potential use as antiarrhythmic agents.

**5.5 Antidysrhyhmic effects of compounds**

The study has shown that the triterpenoids have antiarrhythmic effects on both chemically induced arrhythmias and ischaemia-reperfusion-induced arrhythmias. The study demonstrated that the triterpenoids had an antiarrhythmic effect against adrenaline-induced arrhythmia. The protective effect was comparable to that of propranolol, class II antiarrhythmic agent. Class II antiarrhythmic drugs exert their anti-arrhythmic effects by either reducing or blocking the electrophysiological effects of catecholamines that are mostly mediated by an increase in the slow $Ca^{2+}$ inward current (Vogel and Vogel, 1997). Thus, in the fashion of class II antiarrhythmic drugs, the triterpenoids conferred their

Class IV agents block slow $Ca^{2+}$ channels thereby suppressing the slow $Ca^{2+}$ inward current and calcium dependent slow action potentials (Vaille, *et al.*, 1992; Vogel and Vogel, 1997). This seems to suggest that UA and MM conferred the antiarrhythmic effect by blocking slow calcium inward currents that would have caused re-entry wavelets between the ischaemic areas and the non-ischaemic areas. The blocking of the calcium currents would in turn protect against reperfusion damage that is caused by calcium overload. On the other hand, UV and OA conferred a protective effect in the ischaemia-reperfusion induced arrhythmia that were comparable to that of propranolol, a typical class II antiarrhythmic agent. This indicates that UV and OA conferred their protective effect by a β-adrenergic blocking activity.

The triterpenoids might have also conferred their protective effect on the ischaemia-reperfusion induced arrhythmia through their antioxidant activity. Recent reports have shown that triterpenoids have antioxidant activity (Balanehru and Nagarajan, 1991; Kitani *et al.*, 1999; Zhang *et al.*, 2001; Somova, *et al.*, 2003a and 2003b). It has been reported that antioxidants and calcium antagonists can mitigate injuries caused by reperfusion (Jeroudi *et al.*, 1994). There also reports that have that diminished superoxide radical production of circulating neutrophils during reperfusion has beneficial effects on tissue injury caused especially by free radicals (Roth, *et al.,* 1997). Furthermore, Pataki, *et al.*, (2002) observed that grape seed proanthocyanidins have a protective effect against reperfusion-induced injury via their ability to reduce or remove or even inhibit the formation of free radicals in myocardium. In light of the role free radicals have on reperfusion arrhythmias, it can be postulated that our triterpenoids conferred an antiarrhythmic effect against ischaemia-reperfusion induced arrhythmia through their antioxidant activity.

In terms of the crude ethyl acetate extracts, the study has demonstrated that *Psidium guajava* (hybrid), *Eugenia jambolana, Prunus africana* and *Terminalia riparia* displayed a protective action against adrenaline-induced arrhythmia. The protective effect was comparable to that of propranolol, class II antiarrhythmic agent. On the other hand, *Psidium guajava* (white fruit), *Syzygium cordatum* and *Buddleja salviifolia* displayed an antiarrhythmic effect against a $CaCl_2$-induced arrhythmia. The protective effect was comparable to that of amiodarone, class III antiarrhythmic agents. What is interesting about the antiarrhythmic effects of these phytochemicals is that they belong to class II and class III agents just as it was observed with the triterpenoids. Also of note is the fact that with the exception of *Terminalia riparia*, these plants were the ones that yielded the highest percentage of triterpenoids. Thus, it can be postulated that the antiarrhythmic effects of these phytochemicals was partly due to the presence of these triterpenoids. A quick Medline search showed that this is the first time antiarrhythmic effects of the above plants has been reported.

**5.6 Hypoglycaemic activity of the phytochemicals**

Ethyl acetate crude extracts from *Psidium guajava* (small fruit) *Psidium guajava* (white fruit), *Clerodendrum capense, Terminalia sericea* and *Tetradenia riparia* displayed a potent hypoglycaemic activity. The most potent activity of these plants was that of Psidium guajava (white fruit). Hypoglycaemic activity of the isolated triterpenoids was not done in this study but results from chronic studies have shown that triterpenoids have potent hypoglycaemic activity (Liu *et al.*, 1994; Matsuda *et al.*, 1998; Yoshikawa and Matsuda, 2000; Taniguchi *et al.*, 2002; Somova et l., 2003a and 2003b). It can be postulated that triterpenoids might have the potential to contribute to the overall reduction

of the blood pressure in insulin-resistant animals. Further studies need to be done in such animals since our rats were not insulin-resistant. This work would be interesting to carry out in relation to diabetes since diabetic patients tend to be hypertensive as well.

## 5.7 Interdependent link between structure of compounds and the observed cardiovascular effects

Our results suggest that the triterpenoids have specific binding sites since slight changes in the structure of it affected the potency of the compound. For instance, OA was shown to be more potent than the other four triterpenoids despite the fact that UA is its isomer. Also UV was more potent at decreasing blood pressure than UA despite the fact that UV has the same structure like UA except a change of the carbonylic acid to its primary alcohol. On the other hand, MM did not have any effect save the antiarrhythmic ones though it is an isomer of OA that has the carbonylic acid substituted by an ether. This might point to the fact that specific binding sites mediate the cardiac effects of our triterpenoids just as was shown in the diterpene, forskolin (Seamon *et al.*, 1983; Schmidt and Kukovetz, 1989). These authors showed that modifications made on forskolin structure affected its potency on its cardiac function since derivatives of it with some modifications fail to have any effect neither on isolated heart preparations nor in activating adenylate cyclase. They attributed these varying potencies to the decrease in the binding capacity of the analogous to the specific binding site of Forskolin. On the other hand, particular side-chain modification of forskolin enhances the selectivity for the cardiac isoform stimulation (Toya, Schwencke and Ishikawa, 1998). The present study has shown that modifications made to the triterpenoids derivatives affected their activity. Thus, there is interdependent link between the chemical structures of the triterpenoids isolated from the African

medicinal plants and specific cardiovascular biological activity like hypotensive, diuretic, cardiotonic and antiarrhythmic

## 5.8 FUTURE WORK AND RECOMMENDATIONS

I. Since it is common practice that people combine traditional medicine with western medicine, studies should be carried out to investigate the combine effects of our test compounds with those of the known drugs used to treat hypertension and its associated complications.

II. There is need to check whether the triterpenoids have any direct effect on the vascular smooth muscles. In addition, studies can be carried out to check whether the triterpenoids have any effect on the blood flow

III. There are reports that have showed that prolonged administration of some drugs causes tolerance that results in abolishment of their therapeutic properties. For instance, Papp *et al.*, (1966), demonstrated that propranolol was ineffective against $CaCl_2$-induced fibrillation when it was administered for five days. Thus, studies should investigate whether a prolonged administration of the triterpenoids would decrease their potency.

IV. The mechanisms of the positive inotropic effect of the test compounds need to be studied further by using selective agonists and antagonists of camp, adenylate cyclase and phosphodiesterase.

V. To elucidate the mechanism of the inotropic effect, experiments on the skinned myocardial fibres would be very important. Skinned fibres are deprived of functional intracellular and pericellular membrane systems making them easily permeable to ions and cellular metabolites (Herzig *et al.*, 1981). Furthermore, skinned fibres do not have the capability to store and release $Ca^{2+}$ ions nor for producing and accumulating metabolites. As such, skinned fibres can be used to study the influence of our triterpenoids on the contractile apparatus under the conditions of constant intracellular calcium concentration without any disturbances from other cellular metabolites like ATP and cAMP.

VI. Lastly, the experiments involving the guinea pig atria should be repeated since in bioassays a large number of experiments are required to obtain conclusive results. If such experiments are done, there is a need to increase the number of specific blocking tools used to investigate possible mechanisms involved in the inotropic actions so that the involvement of other receptors is investigated. For instance, the involvement of opiod receptors should be investigated using naloxone and other opiod receptor antagonists

# 6.0 CONCLUSION

The study has reconfirmed that many of the medicinal plants used in Southern Africa contain triterpenoids as part of their chemical constituents. Of the 16 selected and screened plants, *Syzygium cordatum* and *Buddleja salviifolia* crude ethyl acetate extracts yielded the highest percentage of the triterpenoids. The triterpenoids that were isolated and purified from the plants were oleanolic acid, ursolic acid, uvaol and methyl maslinate with oleanolic acid being the most abundant of them. All the isolated triterpenoids and the crude extracts were shown to have a low toxicity.

The study has demonstrated that the four triterpenoids have blood pressure reducing activity in rats. All the phytochemicals reduced blood pressure in the acute experiments whilst in the chronic experiments all the phytochemicals except those from *Psidium guajava* (pink fruit), *Psidium guajava* (hybrid) and *Terminalia sericea* reduced blood pressure. This vasodepressor activity was attributed to the natriuretic and diuretic effect that was exhibited by the triterpenoids. The natriuretic and diuretic effects were comparable, and in a few cases better than those of hydrochlorothiazide. In addition to this hypotensive effects, there are reports that have shown that triterpenoids have antiatherosclerotic (Somova *et al.*, 2003a and 2003b); anti-diabetogenic (Liu *et al.*, 1994; Matsuda *et al.*, 1998; Yoshikawa and Matsuda, 2000; Taniguchi *et al.*, 2002); anti-oxidant (Balanehru and Nagarajan, 1991; Kitani *et al.*, 1999; Zhang *et al.*, 2001; Somova *et al.*, 2003a and 2003b); anti-hyperlipidemic (Ma, 1986; Liu *et al.*, 1987; Somova *et al.*, 2003a and 2003b). Thus, triterpenoids have a high potential of being used for the treatment of hypertension due to its action on the variety of factors that cause or are associated with the development of it.

The study has also shown that our triterpenoids have a positive inotropic effect on isolated guinea pig atria and in anaesthetised rats. The inotropic effect was shown to be mediated through β-adrenergic activity. The triterpenoids, except MM, were also able to potentiate the positive inotropic effect of isoprenaline. However, the study did not demonstrate the mechanism through which this potentiating effect was achieved, and this needs further study.

The other major point that has come out of this study is that triterpenoids have an antiarrhythmic effect against adrenaline-induced arrhythmia, $BaCl_2$-induced arrhythmia, and ischaemia-reperfusion induced arrhythmia but not against $CaCl_2$-induced arrhythmia. In terms of the adrenaline-induced arrhythmia, the protective effect of the triterpenoids was similar to that of a class II antiarrhythmic agent whilst for the $BaCl_2$-induced arrhythmia the protective effect was comparable to that of a class III antiarrhythmic agent. The triterpenoids offered a protective effect on ischaemia/reperfusion-induced arrhythmia in a manner that was comparable to class II and IV antiarrhythmic agents. Furthermore, the study postulated that the triterpenoids conferred a protective effect on reperfusion-induced arrhythmia most probably through their antioxidant activity.

The study has demonstrated that there is an interdependent link between the chemical structures of the triterpenoids isolated from the African medicinal plants and specific cardiovascular biological activity like hypotensive, diuretic, cardiotonic and antiarrhythmic. The triterpenoids that were isolated and used in this study displayed different potencies when subjected to the pharmacological analysis despite the fact that they are isomers of each other. Thus, the study has shown that there is a link between the chemical structure of the triterpenoids and its cardiovascular activity.

Therefore, evidence provided in this study indicated that triterpenoids have hypotensive, cardiotonic and antiarrhythmic activities. Since the triterpenoids have been demonstrated to be present in many of the medicinal plants of Southern Africa, the triterpenoids would provide a cheap and accessible traditional medicine source for the treatment of complicated hypertension in Southern Africa.

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# 8.0 APPENDICES

## APPENDIX 1

**Recipes**

**Krebs-Hanseleit Buffer (pH 7.4)**

| Components | In mM | FW | mmol/L | mM/5L |
|---|---|---|---|---|
| NaCl | 118.00 | 58.44 | 0.05844 x 118= 6.896g | 34.480g |
| KCl | 4.70 | 74.55 | 0.07455 x 4.7= 0.350g | 1.752g |
| $KH_2PO_4$ | 1.18 | 136.09 | 0.13609 x 1.18 = 0.160g | 0.803g |
| $MgSO_4.7H_2O$ | 1.17 | 246.48 | 0.24648 x 1.17 = 0.288g | 1.442g |
| $CaCl_2.2H_2O$ | 1.60 | 147.02 | 0.14702 x 1.6 = 0.235g | 1.176g |
| $NaHCO_3$ | 25.00 | 84.01 | 0.08401 x 25 = 2.100g | 10.501g |
| Glucose | 11.10 | 180.16 | 0.18016 x 11.1= 1.999g | 10.000g |

* $KH_2PO_4$ and $MgSO_4.7H_2O$ were added last whilst glucose was added on the day of the experiments.

# APPENDIX 2

**Weights of crude extracts from different plants**

Table 18. Weight (g) of crude hexane extracts

| Name of plant | Code | Weight of extract (g) |
|---|---|---|
| Psidium guajava (small fruit) | MH/01/A | 2.5 |
| Psidium guajava (white fruit) | MH/02/A | 2.564 |
| Psidium guajava (Hybrid) | MH/03/A | 3.74 |
| Centella asiatica | MH/04/A | 1.239 |
| Syzygum cordatum | MH/05/A | 1.239 |
| Psychotreia serpeus | MH/06/A | 1.501 |
| Syzygum cordatum | MH/07/A | 10.143 |
| Eugenia jambulana | MH/08/A | 13.323 |
| Clerodendreum l. | MH/09/A | 18.254 |
| Plantago major | MH/10/A | 10.71 |
| Psidium guajava (pink fruit) | MH/11/A | 4.425 |
| Plantago lanceolata | MH/12/A | 6.856 |
| Terminalia sericea | MH/13/A | 10.145 |
| Prunus africana | MH/14/A | 10.456 |
| Combretum molle | MH/15/A | 8.774 |
| Buddleja s. | MH/16/A | 2.768 |
| Centella asiatica | MH/17/A | 4.685 |
| Tetradenia | MH/18/A | 11.002 |

Table 19. Weight (g) of Dichloromethane crude extracts

| Name of plant | Code | Weight of extract (g) |
|---|---|---|
| Psidium guajava (small fruit) | MH/01/B | 5.971 |
| Psidium guajava (white fruit) | MH/02/B | 4.33 |
| Psidium guajava (Hybrid) | MH/03/B | 4.201 |
| Centella asiatica | MH/04/B | 1.17 |
| Syzygum cordatum | MH/05/B | 4.075 |
| Psychotreia serpeus | MH/06/B | 8.222 |
| Syzygum cordatum | MH/07/B | 29.279 |
| Eugenia jambulana | MH/08/B | 22.008 |
| Clerodendreum l. | MH/09/B | 32.784 |
| Plantago major | MH/10/B | 7.678 |
| Psidium guajava (pink fruit) | MH/11/B | 20.247 |
| Plantago lanceolata | MH/12/B | 18.589 |
| Terminalia sericea | MH/13/B | 13.243 |
| Prunus africana | MH/14/B | 21.481 |
| Combretum molle | MH/15/B | 7.276 |
| Buddleja s. | MH/16/B | 7.370 |
| Centella asiatica | MH/17/B | 3.231 |
| Tetradenia | MH/18/B | 5.328 |

Table 20. Weight (g) of crude ethyl acetate extract

| Name of plant | Code | Weight of extract (g) |
|---|---|---|
| Psidium guajava (small fruit) | MH/01/C | 6.892 |
| Psidium guajava (white fruit) | MH/02/C | 5.378 |
| Psidium guajava (Hybrid) | MH/03/C | 3.116 |
| Centella asiatica | MH/04/C | 0.32 |
| Syzygum cordatum | MH/05/C | 2.11 |
| Psychotreia serpeus | MH/06/C | 8.089 |
| Syzygum cordatum | MH/07/C | 25.286 |
| Eugenia jambulana | MH/08/C | 14.860 |
| Clerodendreum l. | MH/09/C | 10.152 |
| Plantago major | MH/10/C | 3.886 |
| Psidium guajava (pink fruit) | MH/11/C | 8.854 |
| Plantago lanceolata | MH/12/C | 6.894 |
| Terminalia sericea | MH/13/C | 5.247 |
| Prunus africana | MH/14/C | 34.874 |
| Combretum molle | MH/15/C | 3.687 |
| Buddleja s. | MH/16/C | 5.955 |
| Centella asiatica | MH/17/C | 2.026 |
| Tetradenia | MH/18/C | 1.016 |

Table 21. Weight (g) of crude methanol extracts

| Name of plant | Code | Weight of extract (g) |
|---|---|---|
| Psidium guajava (small fruit) | MH/01/D | 4.244 |
| Psidium guajava (white fruit) | MH/02/D | 5.441 |
| Psidium guajava (Hybrid) | MH/03/D | 5.752 |
| Centella asiatica | MH/04/D | 3.285 |
| Syzygum cordatum | MH/05/D | 8.425 |
| Psychotreia serpeus | MH/06/D | 162.016 |
| Syzygum cordatum | MH/07/D | 137.323 |
| Eugenia jambulana | MH/08/D | 18.312 |
| Clerodendreum l. | MH/09/D | 66.338 |
| Plantago major | MH/10/D | 110.519 |
| Psidium guajava (pink fruit) | MH/11/D | 14.841 |
| Plantago lanceolata | MH/12/D | 114.771 |
| Terminalia sericea | MH/13/D | 63.265 |
| Prunus africana | MH/14/D | 187.910 |
| Combretum molle | MH/15/D | 36.612 |
| Buddleja s. | MH/16/D | 16.156 |
| Centella asiatica | MH/17/D | 61.049 |
| Tetradenia | MH/18/D | 8.245 |

## APPENDIX 3- PICTURES OF EXPERIMENTAL SET UP

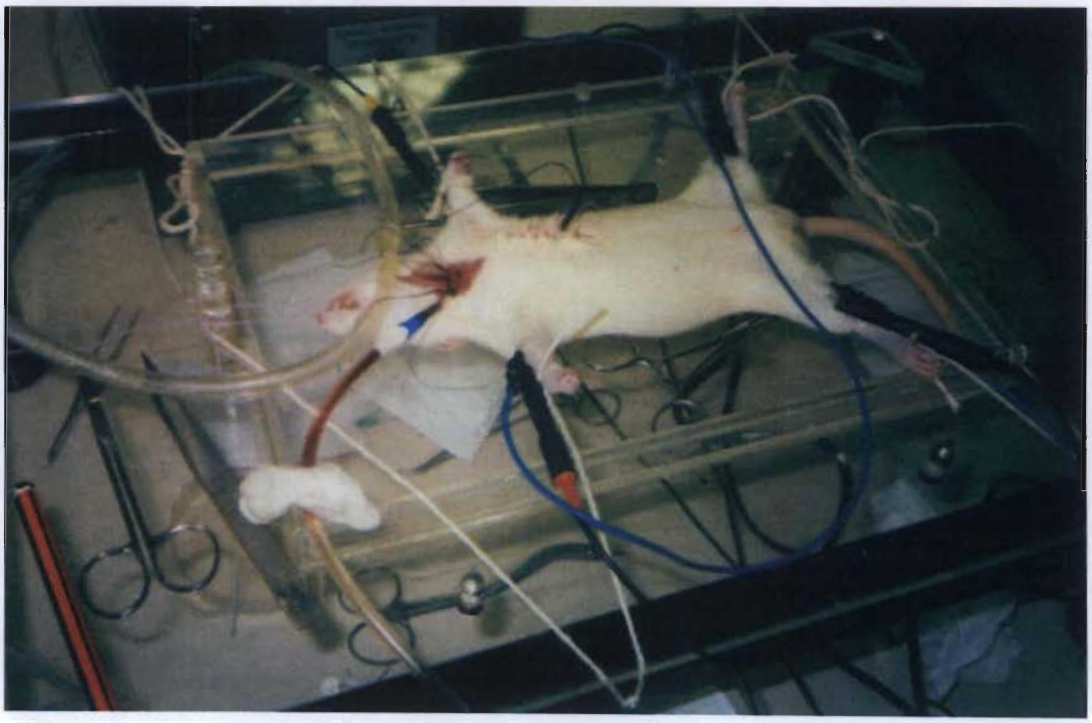


Figure 35. Scanned picture of the experimental set up for induction of ischaemia and reperfusion

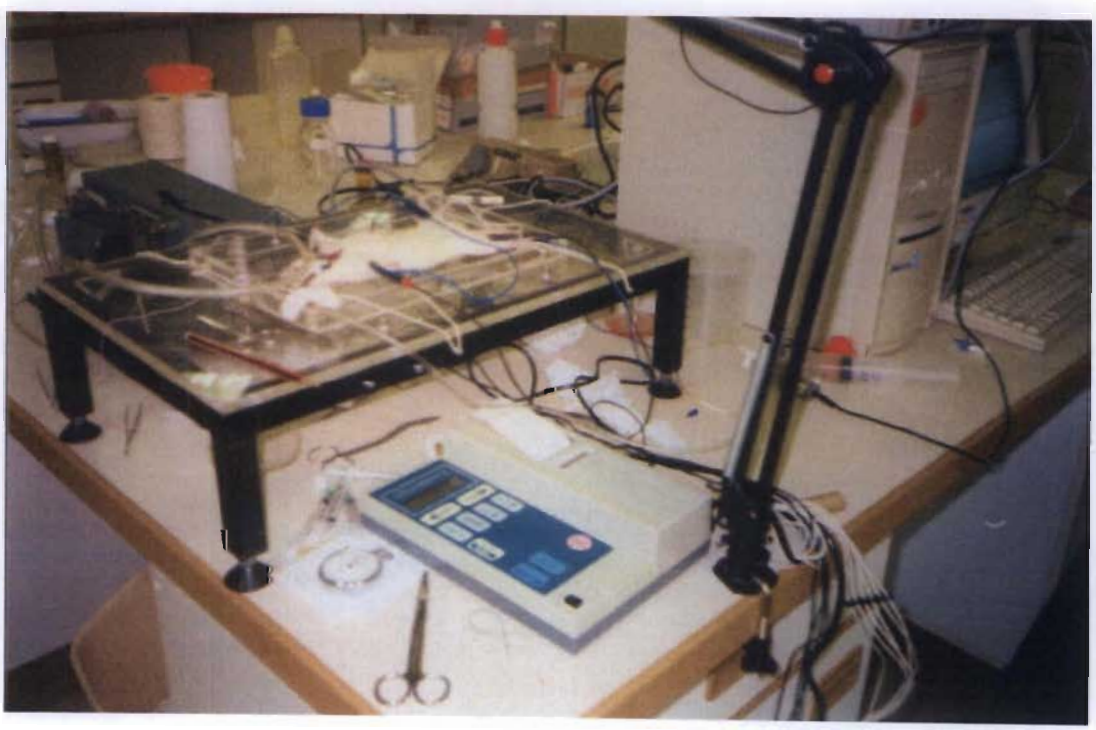


Figure 36. Scanned picture of the experimental set up for induction of ischaemia and reperfusion . The Powerlab system, ECG and rodent ventilator are shown.

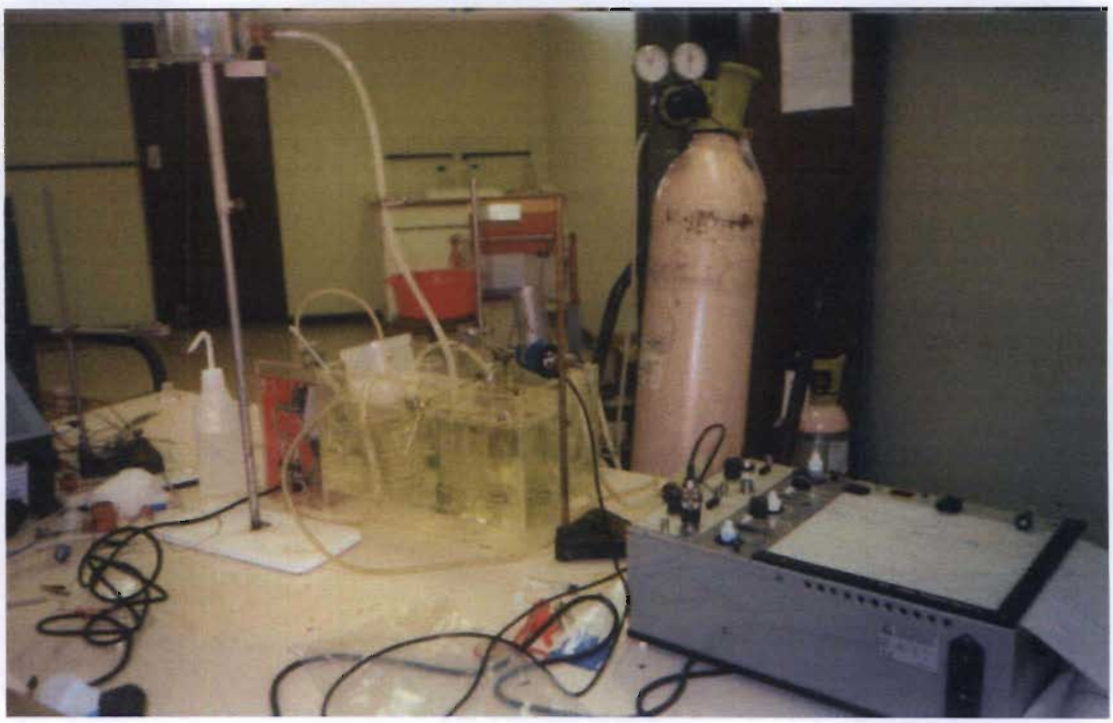


Figure 37. Scanned pictures of the experimental system for cardiotonic studies

## APPENDIX 4

**Representative ECGs from the analysis of chemically induced arrhythmia**

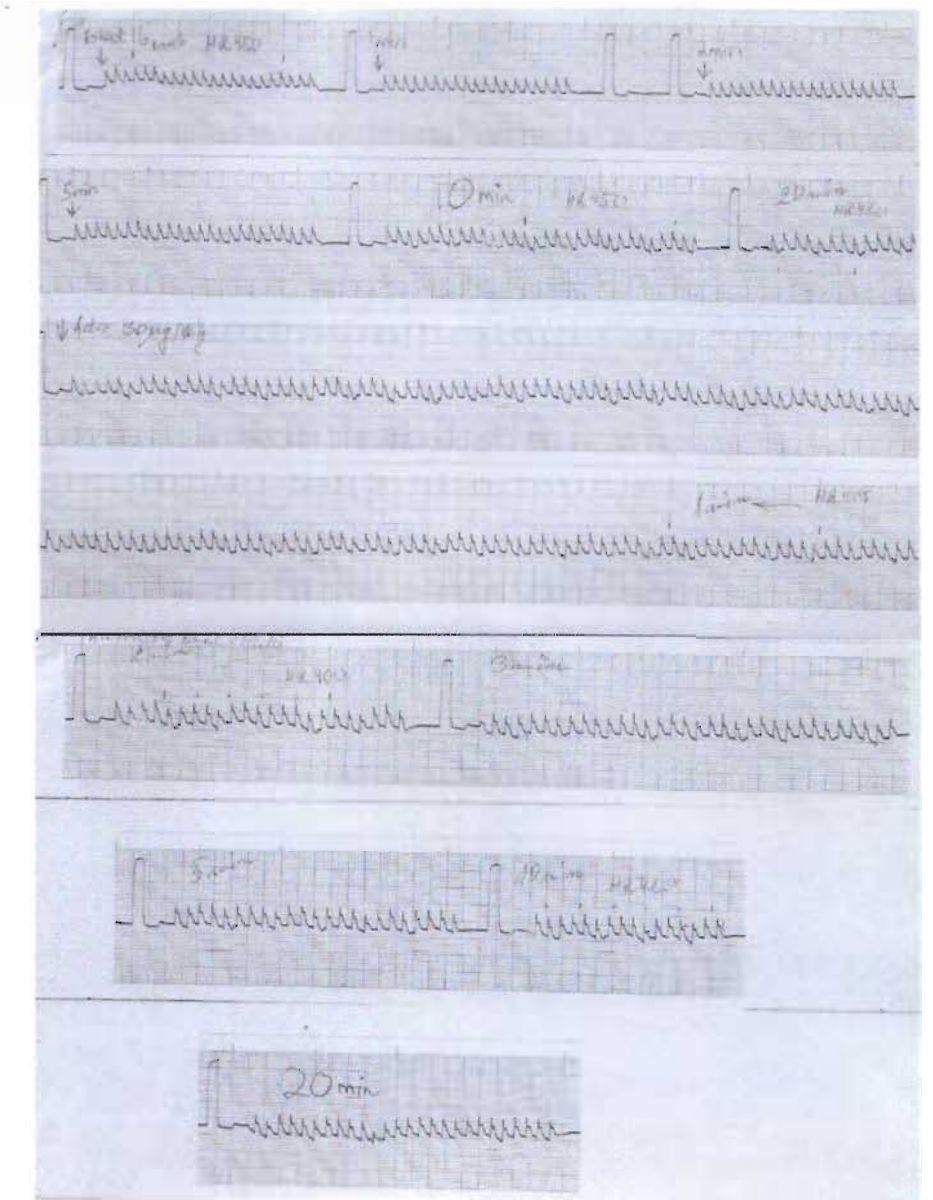



Figure 38. ECG of adrenaline on the background of *Psychotria serpens*

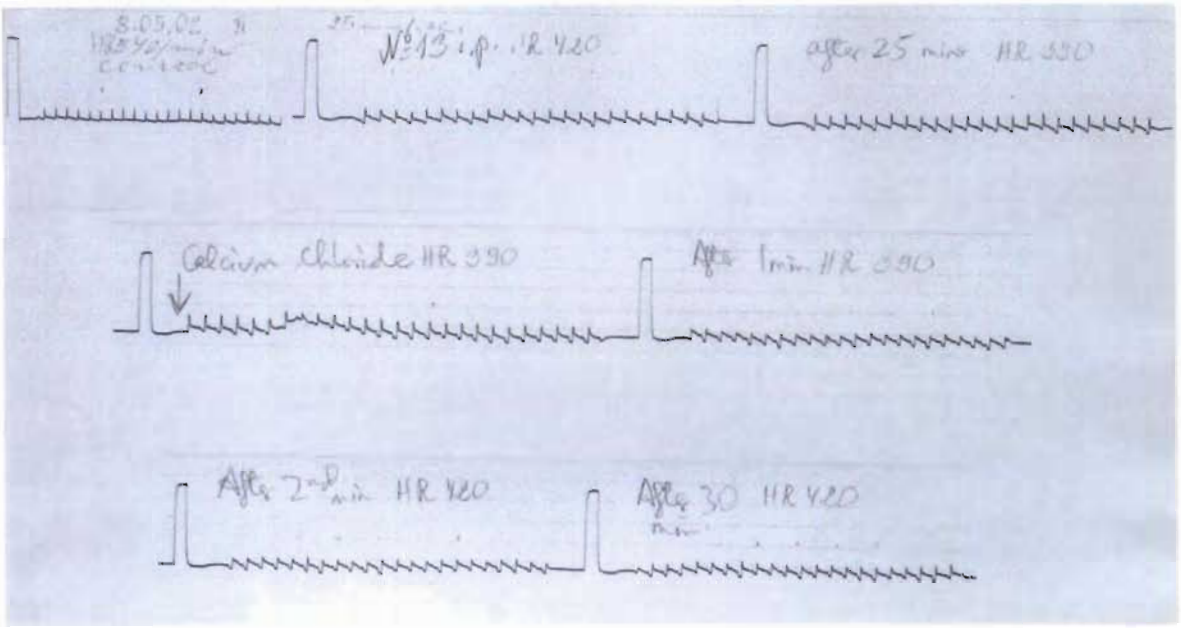



Figure 39. ECG of $CaCl_2$ on the background of *Buddleja salviifolia*

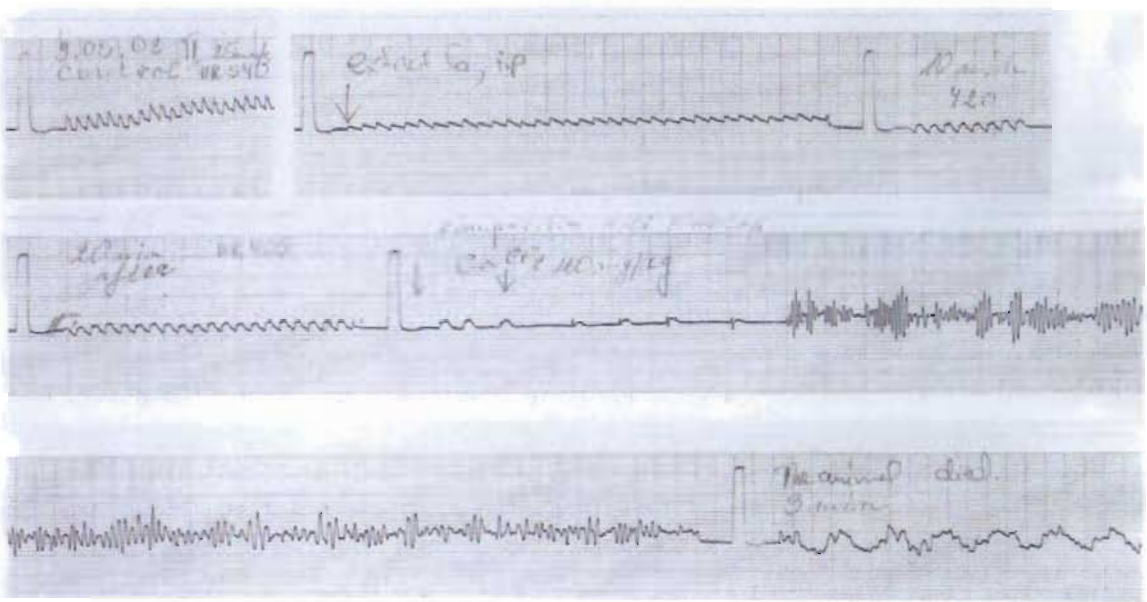


Figure 40. ECG of $CaCl_2$ on the background of *Eugenia jambolana*

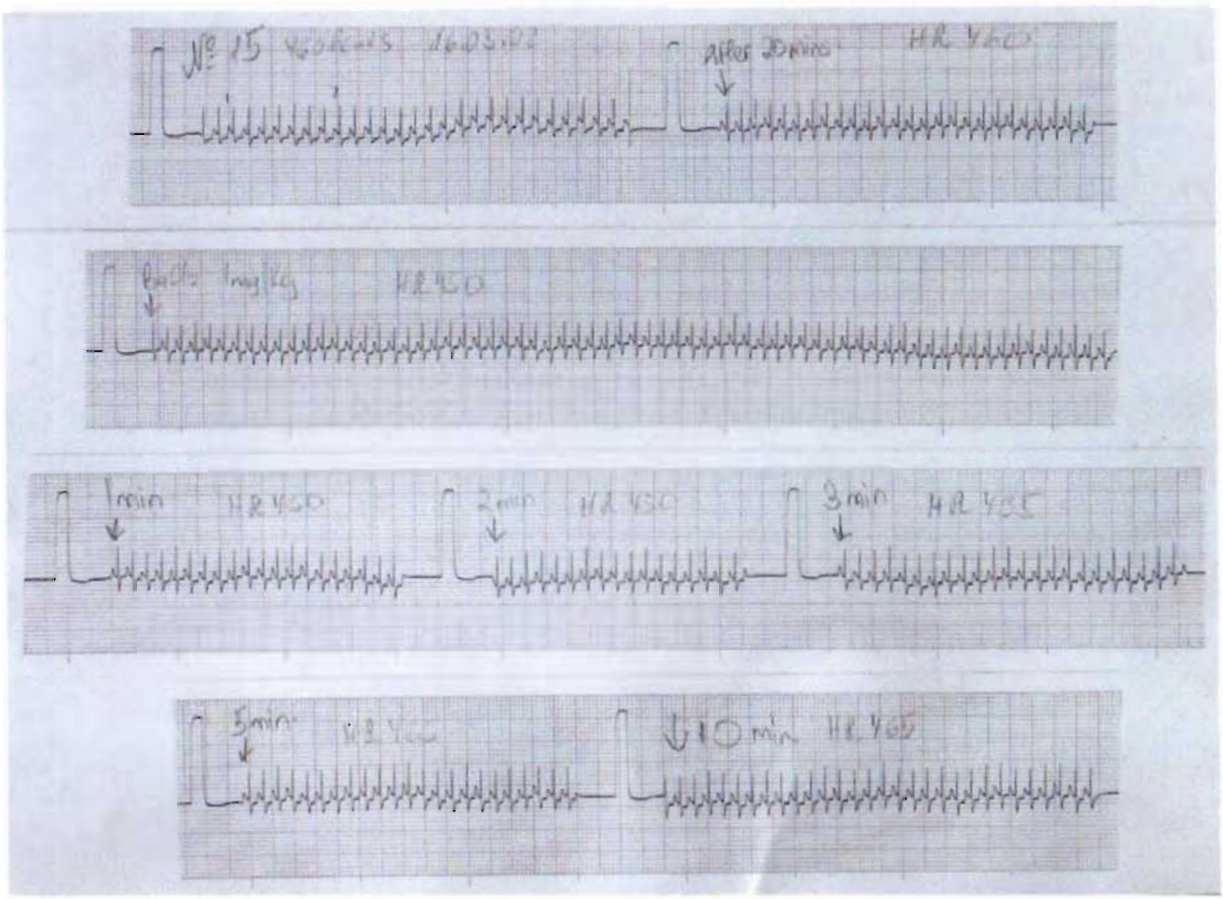


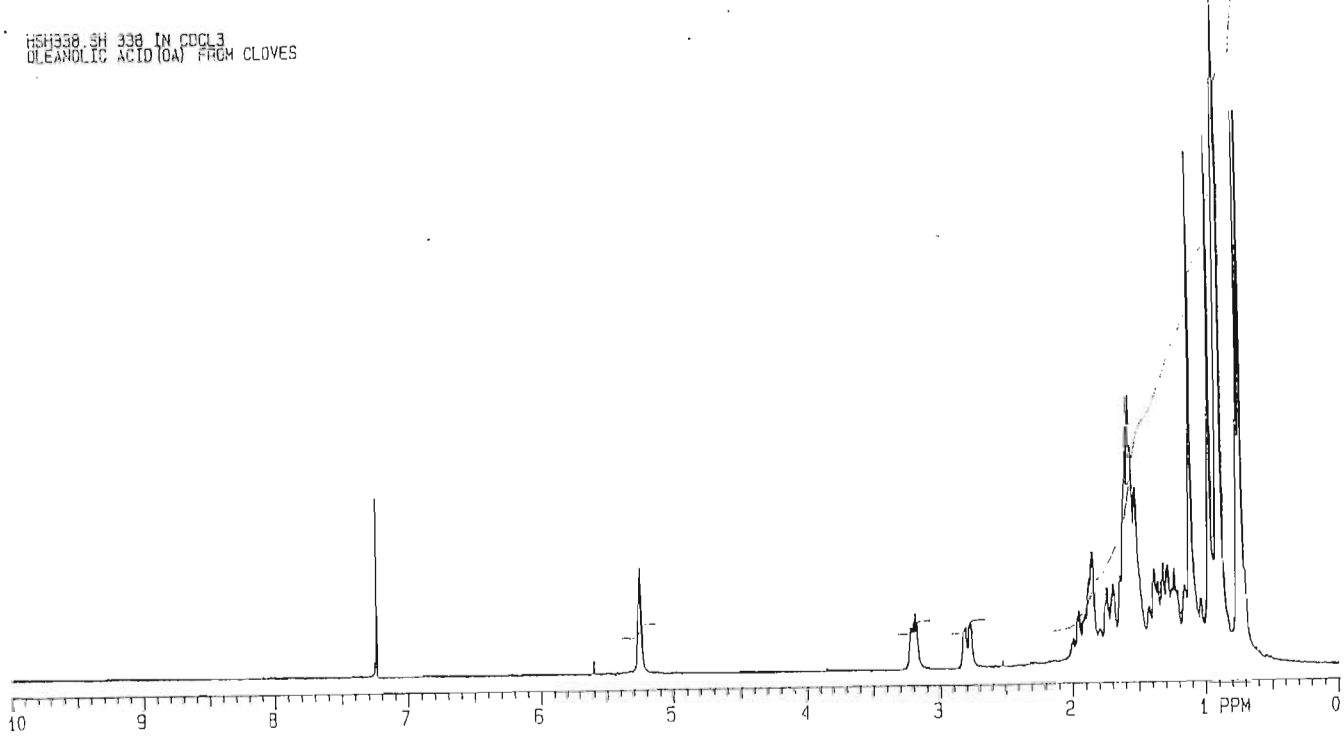
Figure 41. ECG of $BaCl_2$ on the background of *Tetradenia riparia*

# APPENDIX 5

## Spectral data of the isolated compounds

Table 22: Chemical composition of the isolates and their plant source

| Spectral number | Isolate code | Plant source | Chemical composition |
|---|---|---|---|
| SH 366 | MH/07/B | *Syzygium cordatum* | Oleanolic acid (major) and betulinic acid (minor) |
| SH 367 | MH/09/C | *Clerodendreum trichotomum* | Betulinic acid |
| SH 372 | MH/07/C | *Syzygium cordatum* | Oleanolic and ursolic acids |
| SH 377 | MH/07/D | *Syzygium cordatum* | Ursolic acid (major) |
| SH 398 | MH/08/C9 | *Eugenia jambolana* | Ursolic acid |
| SH 408 | PM/15/F | *Prunus africana* | Ursolic acid |
| SH 413 | PM/09/G | *Plantago major* | Oleanolic and ursolic acids |



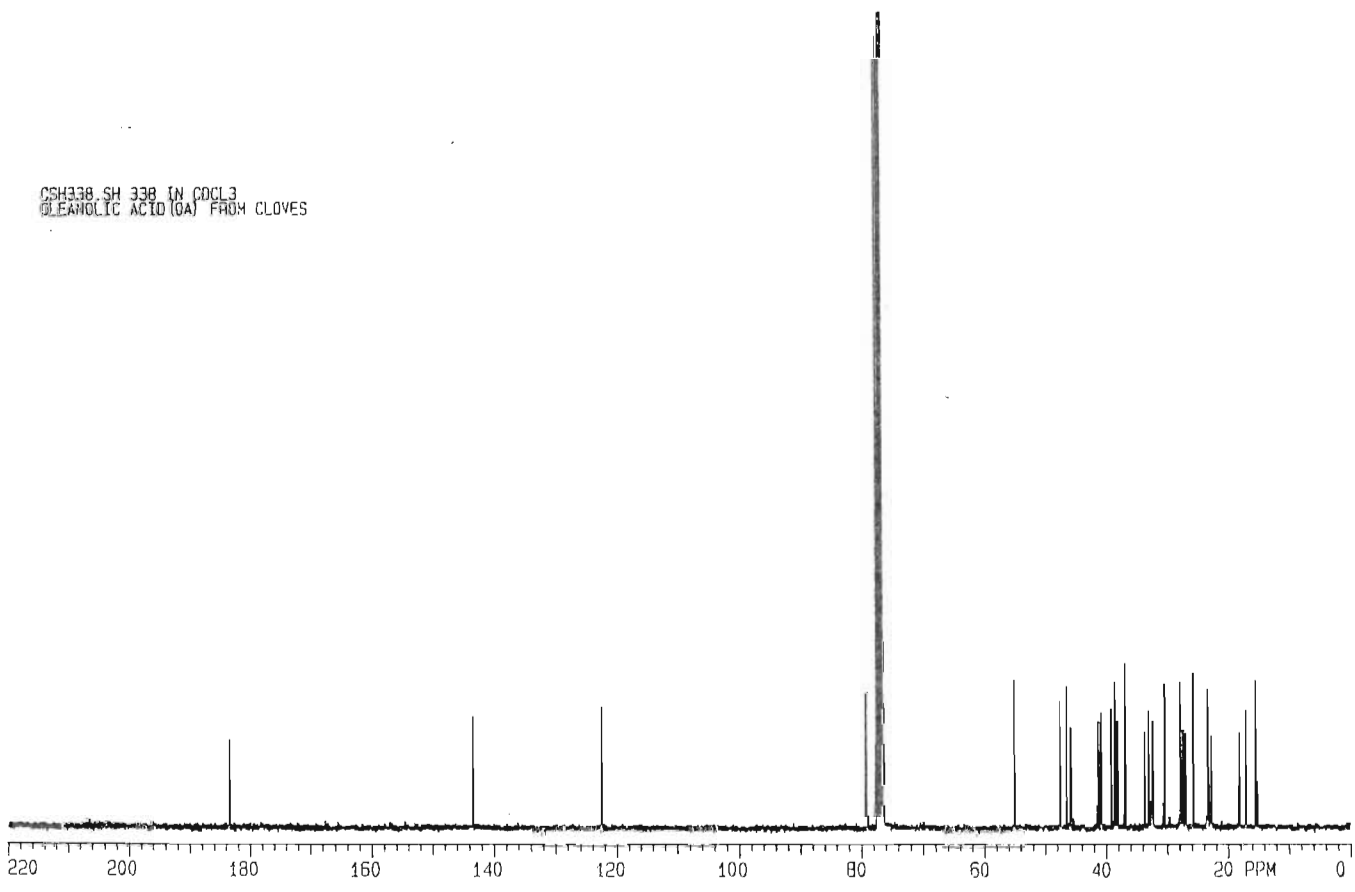




Fig. 42. Spectral data of authentic oleanolic acid

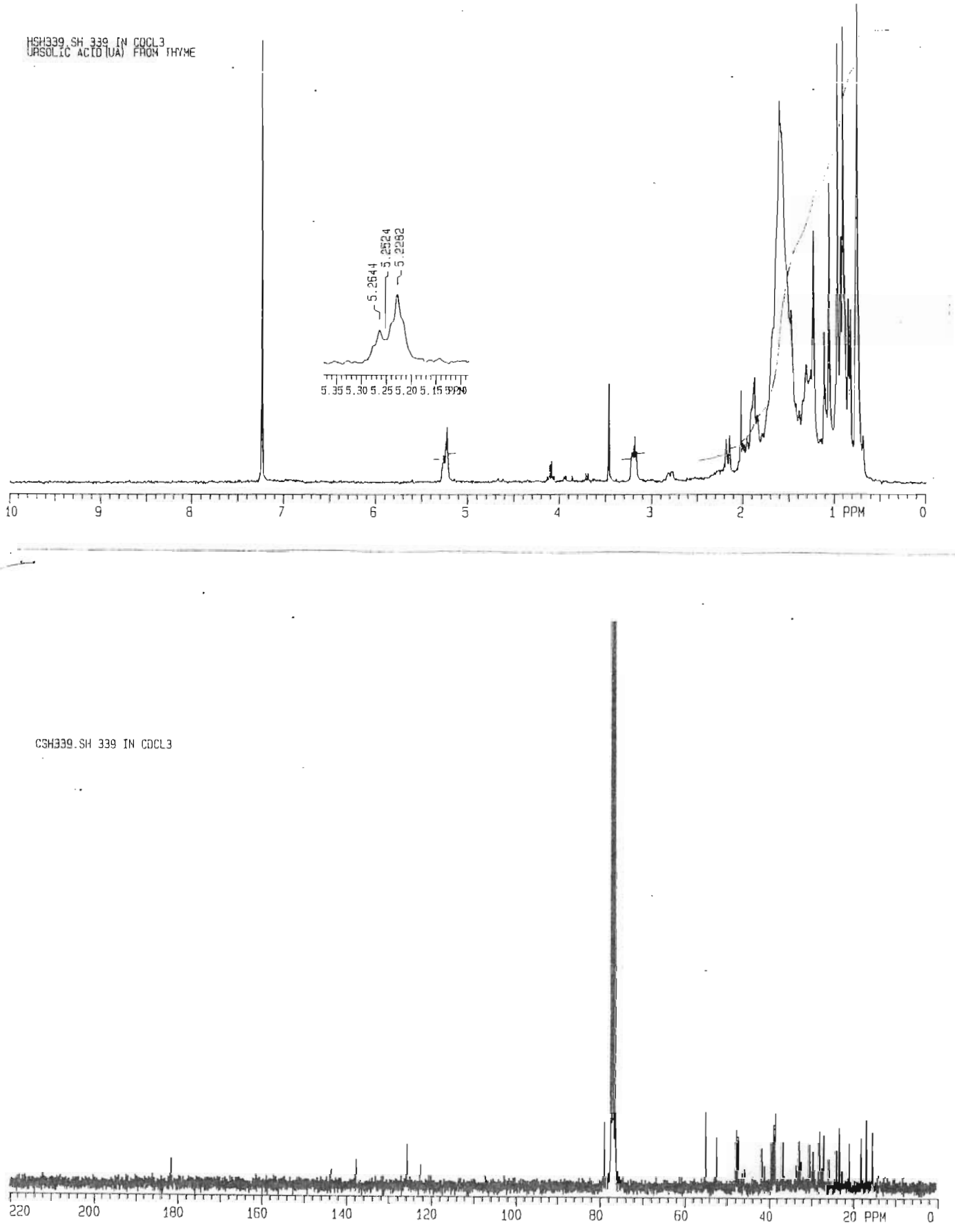
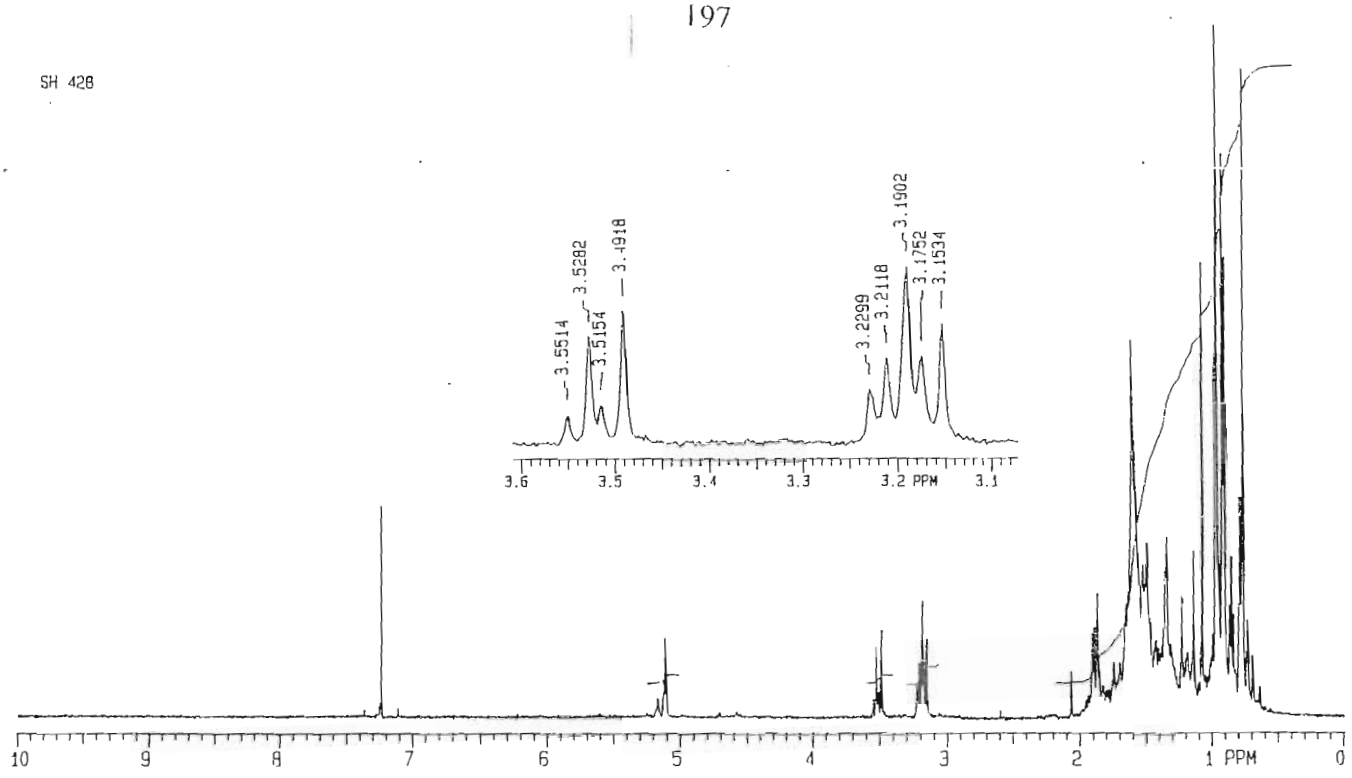






Fig. 43. Spectral data of authentic ursolic acid



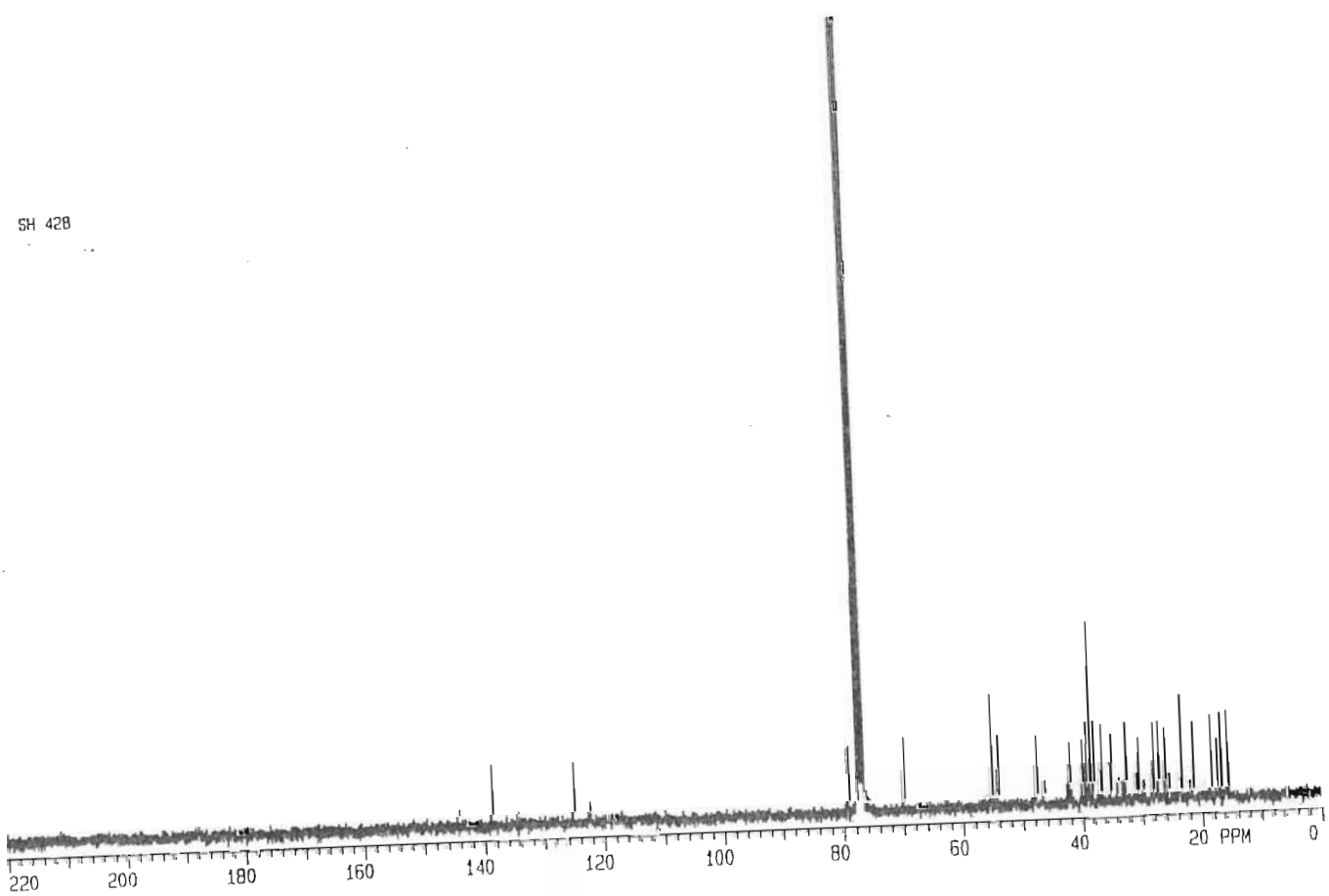
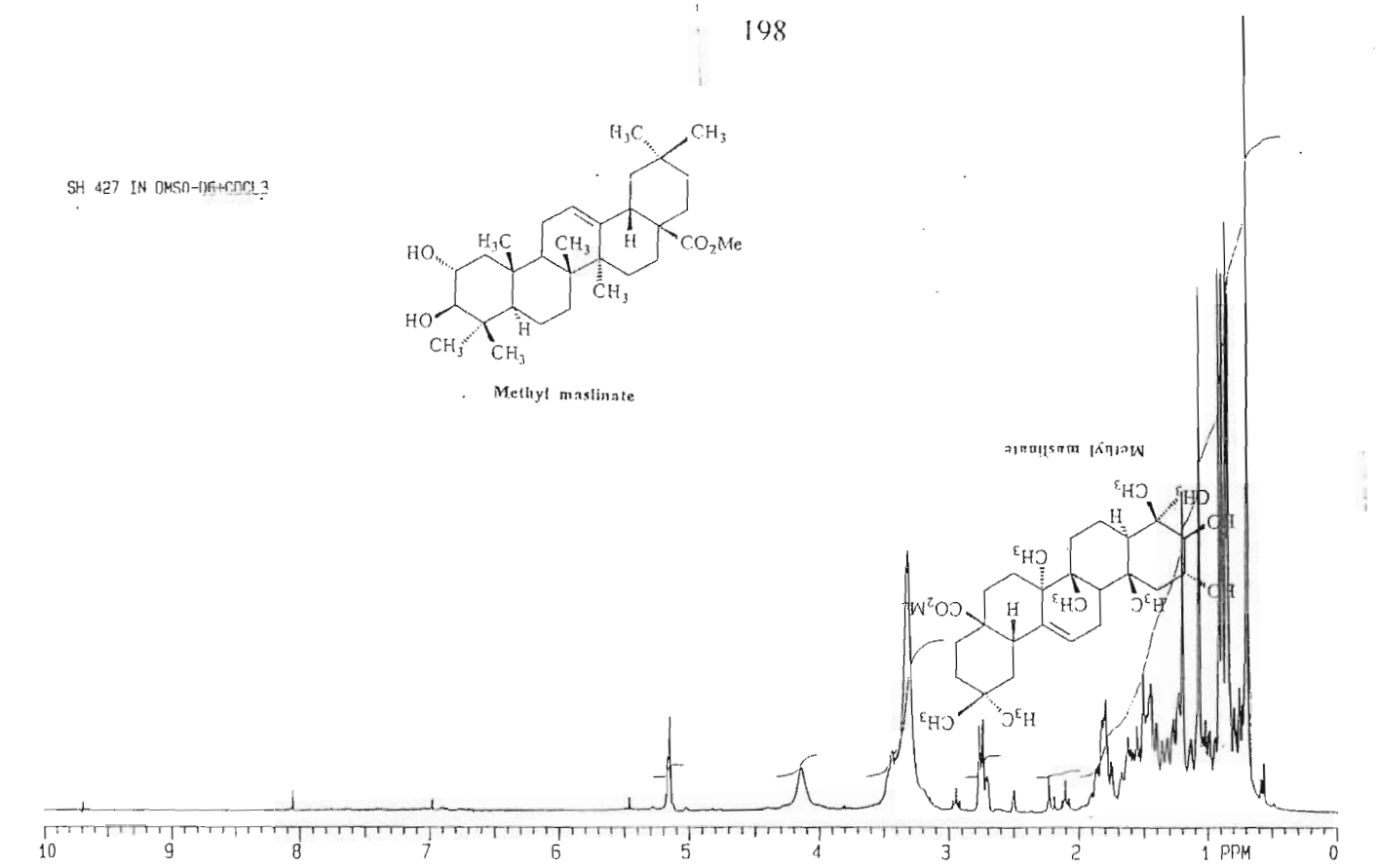




Fig. 44. Spectral data of authentic uvaol



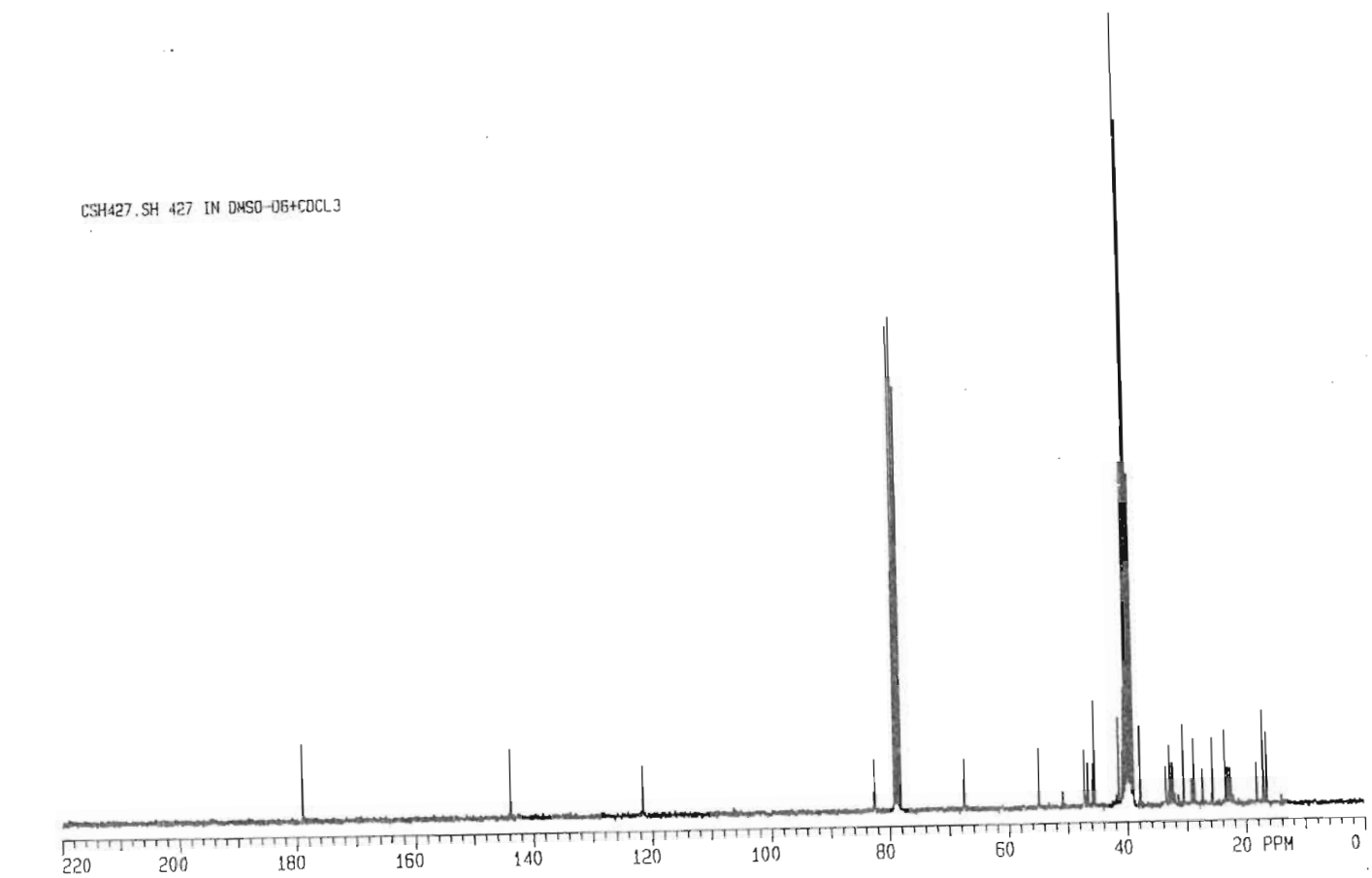
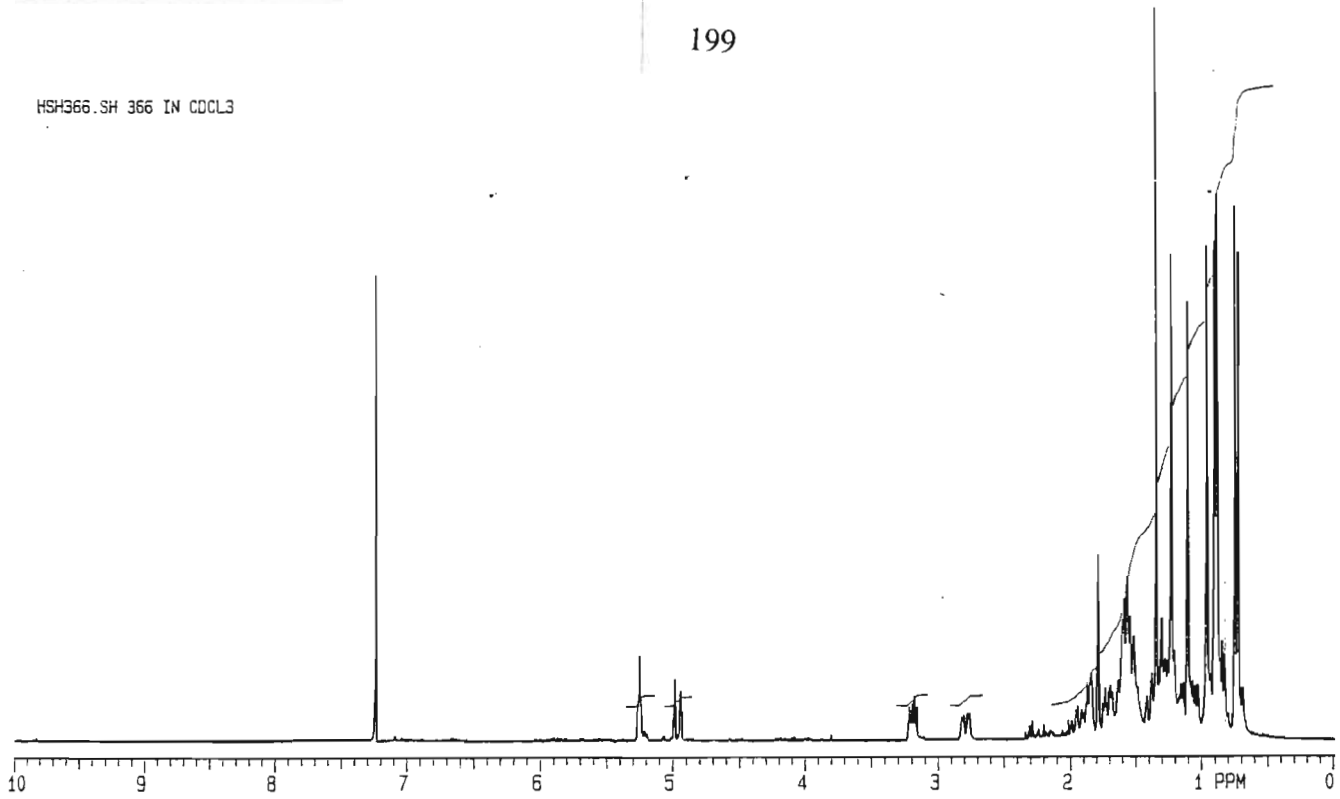




Fig. 45. Spectral data of authentic methyl maslinate



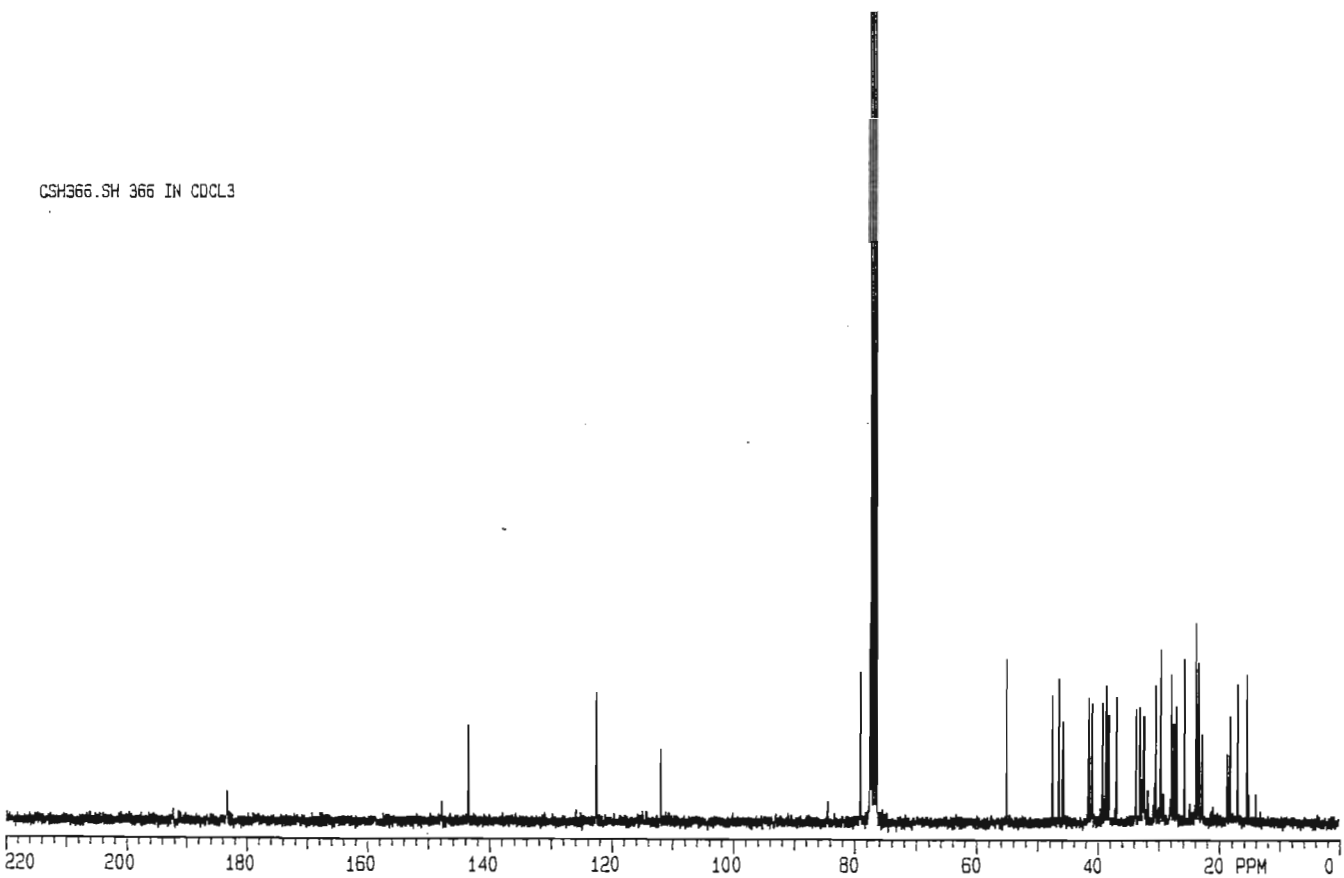




Fig. 46. Spectral data of MH/07/B

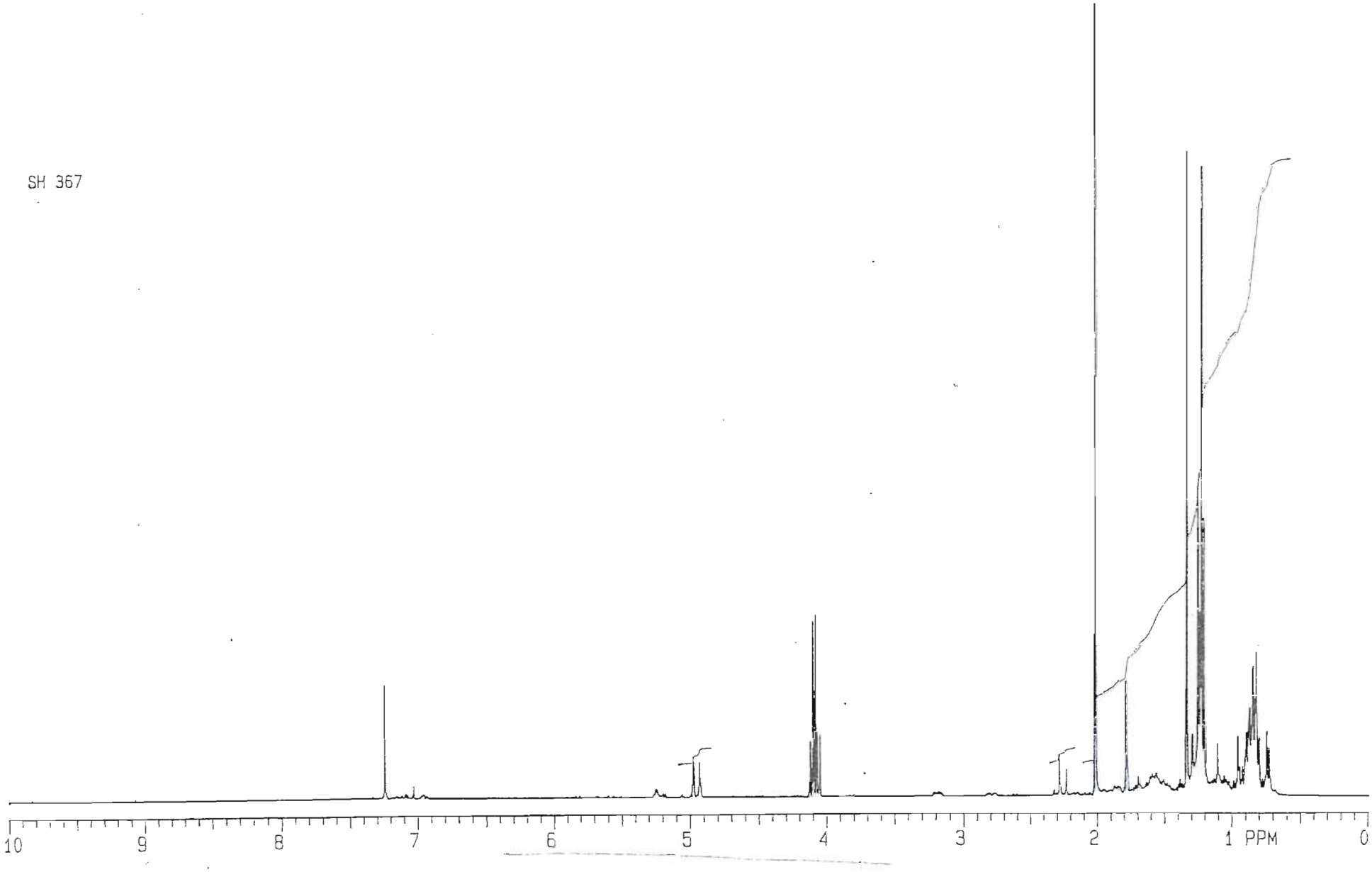



Fig. 47. Spectral data of MH/09/C