PHARMACOLOGICAL EVALUATION OF THE MEDICINAL PLANTS, *PELARGONIUM TRISTE* (L), *ELYTROPAPPUS RHINOCEROTIS* (L.F), AND *OLEA EUROPAEA AFRICANA* (MILL.) FOR ANTIDIARRHOEAL ACTIVITY IN MICE

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Thesis submitted in partial fulfilment of the requirements for the degree of Magister Pharmaceuticae, School of Pharmacy, University of the Western Cape.

Supervisor: Prof. George J. Amabeoku

May 2001

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KEYWORDS

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Olea europaea africana (Mill.)

Castor oil

Phytochemical screening

HLPC fingerprints

ABSTRACT

PHARMACOLOGICAL EVALUATION OF THE MEDICINAL PLANTS, *PELARGONIUM TRISTE* (L.), *ELYTROPAPPUS RHINOCEROTIS* (L.F.), AND *OLEA EUROPAEA AFRICANA* (MILL.) FOR ANTIDIARRHOEAL ACTIVITY IN MICE.

KAPINGA BAMUAMBA

M. Pharm. thesis, Department of Pharmacology, School of Pharmacy, University of the Western Cape.

Three medicinal plant species, *Pelargonium triste* (L.), *Elytropappus rhinocerotis* (L.F.), and *Olea europaea africana* (Mill.), commonly used in the Western Cape traditional medicine to treat various ailments were assessed for activity against castor oil-induced diarrhoea in mice. The chemical composition and the high performance liquid chromatographic (HPLC) analysis of the plant extracts were also investigated.

At the doses of 25 mg/kg, 50 mg/kg, and 75 mg/kg all the plant extracts significantly ($p < 0.05$) reduced the number of diarrhoeal episodes in mice. At the doses of 50 mg/kg and 75 mg/kg the *P. triste* and *E. rhinocerotis* aqueous extracts significantly reduced the total diarrhoeal stool mass, and also significantly delayed the onset of diarrhoea in mice. The effect of *P. triste* against castor oil-induced diarrhoea was dose dependent.

Olea europaea africana did not significantly alter the onset of diarrhoea or the total diarrhoeal stool mass.

The data obtained indicate that *P. triste*, *E. rhinocerotis,* and *O. europaea africana* possess anti-diarrhoeal properties, which justify their use in the Western Cape by traditional medicines practitioners to treat diarrhoea. The data also show that all three plant-species contain tannins and saponins. In addition, *P. triste* and *O. europaea africana* contain reducing sugars, where as *E. rhinocerotis* contains cardiac glycosides.

May 2001

DECLARATION

I declare that "Pharmacological evaluation of medicinal plants, *Pelargonium triste* (L.), *Elytropappus rhinocerotis* (L.F.), and *Olea europaea africana* (Mill.) for antidiarrhoeal activity in mice" is my own work, that it has not been submitted for any degree or examination in any other university, and that all the sources I have used or quoted have been indicated and acknowledged by complete references.

Full name.....

Date.....

Signed.....

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Special thanks to my dear wife, Solange and my sons, Chançard, Samuel, and Prince Ntumba, who endured so much during the entire period of the study.

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CHAPTER I

INTRODUCTION

Diarrhoea is one of the commonest diseases and a major cause of death in the world. On a global scale, diarrhoeal diseases are second only to cardiovascular diseases as a cause of death (Mandell at el., 1990). It is reported that 4,5 to 6 millions children die from diarrhoeal diseases each year in Asia, Africa, and Latin America. 13% of Latin American children die of diarrhoea before reaching their first birthday. In USA, the diarrhoea kills about 10,000 people every year (Mandell at el., 1990).

Modern medicine has successfully used the "Oral Rehydration Solution" (ORS) to combat and reduce fatalities due to acute diarrhoea. However, the proportion of ORS users is frustratingly low, especially in rural and semi-urban areas of the Third World. Reasons for this have been attributed mostly to the accessibility of the health care centres that provide the ORS packets. Sugar- salt solution and home- based fluids are the alternatives. But there too, the number of sugar-salt solution users is not encouraging neither, since only few mothers can correctly prepare the solution at home (Rabbani, 2000).

Instead, in many developing countries, indigenous populations rely mainly on the use of herbal remedies from traditional medicine practitioners for their treatment. The use of herbal remedies to treat various ailments is an old practice that still enjoys widespread popularity The Word Health

Organisation believes that approximately 80% of the world's inhabitants are served for primary health care service through this practice (Shale at el., 1999). In South Africa for example, it is estimated that more than 75% of black population consult traditional medicine practitioners (Rajendra, 1995).

However, the empirical character that is typical of traditional medicine systems constitutes a serious problem, with regard to the safety and efficacy of the medications. Too often, the therapeutic properties of traditional herbal remedies are claimed without any scientific prove.

There is therefore an urgency to scientific assess the traditional medicinal plant species. Equally urgent is also the need to further explore the "Mother Nature", flora and fauna, in order to find novel remedies to diseases, including these for which cures have not been discovered such as HIV/ AIDS, asthma, certain types of cancers, and so on.

In this project, three medicinal plant species, *P. triste*, *E. rhinocerotis* and *O. euro. africana*, commonly used in traditional medicine, in the Western Cape, have been investigated for their antidiarrhoeal activity. Their phytochemical constituents and the HPLC analyses were also investigated. The aim of the present study is to validate the claim by traditional medicine practitioners concerning the antidiarrhoeal activity of t these plants.

CHAPTER 2

LITERATURE SURVEY

2.1 DIARRHOEA

2.1.1 What Is Diarrhoea ?

Diarrhoea is loose, watery stools occurring more than three times in one day. It is a common problem that usually lasts a day or two and goes away on its own without any special treatment. However, prolonged diarrhoea can be a sign of other problems.

Diarrhoea can cause dehydration, which means the body lacks enough fluid to function properly. Dehydration is particularly dangerous in children and the elderly, and it must be treated promptly to avoid serious health problems. Dehydration will be discussed in the following pages.

People of all ages can get diarrhoea. The average adult has a bout of diarrhoea about four times a year (NIDDK: National Digestive Diseases information Clearinghouse, 2001)

2.1.2 Symptoms of diarrhoea

Cramps in the lower abdomen

Severe pain or spasms in abdomen

Loose stools

Unformed stools

Watery discharge with bowel movement that may or may not contain mucous

Fever (not always present)

Lack of bowel control in young and elderly

Nausea, weakness, and malaise

2.1.3 Causes of diarrhoea

A temporary problem, such as an infection, or a chronic problem, such as an intestinal disease may cause diarrhoea. Diarrhoea often accompanies flu and similar viral conditions.

The most common causes of diarrhoea include:

Stress

Food intolerance: Some people are unable to digest a component of food, such as lactose, the sugar found in milk.

Food poisoning

Food allergy

Malabsorption Syndrome

Irritable Bowel Syndrome

Excessive alcohol usage

Ingestion of tainted or contaminated water

Lactose Intolerance

Sorbitol Intolerance

Disease of the pancreas

Foods (beans, prunes, orange juice)

Menstrual cycle

Menopause

Unsanitary conditions

Infection: bacterial infections. Several types of bacteria, consumed through contaminated food or water, can cause diarrhoea. Common culprits include *Campylobacter, Salmonella, Shigella,* and *Escherichia coli.*

Viral infections: Many viruses cause diarrhoea, including rotavirus, Norwalk virus, cytomegalovirus, herpes simplex virus, viral hepatitis, echovirus, and HIV.

Parasitic infections: Parasites can enter the body through food or water and settle in the digestive system. Parasites that cause diarrhoea include *Giardia lamblia, Entamoeba histolytica,* and *Cryptosporidium.*

Reaction to medicines, such as antibiotics, blood pressure medications, and antacids containing magnesium.

Fungal infections

Some people develop diarrhoea after stomach surgery or removal of the gallbladder. The reason may be a change in how quickly food moves through the digestive system after stomach surgery or an increase in bile in the colon that can occur after gallbladder surgery (NIDDK: National Digestive Diseases information Clearinghouse, 2001)

People who visit foreign countries are at risk for traveler's diarrhoea, which is caused by eating food or drinking water contaminated with

bacteria, viruses, or, sometimes, parasites. Traveler's diarrhoea is a particular problem for people visiting developing countries.

It must be noted however, that in many cases, the cause of diarrhoea may not be found. As long as diarrhoea goes away on its own, an extensive search for the cause is not usually necessary (NIDDK: National Digestive Diseases information Clearinghouse, 2001)

2.1.4 When to begin treatment

Diarrhoea treatment should never begin immediately upon the onset of symptoms. Diarrhoea is a natural way for the body to rid itself of toxins. Suppressing toxins, especially those caused by food poisoning, can lead to serious complications. The most dangerous complication of diarrhoea is the state of dehydration of the patient. The very young and old are at high risk for developing dehydration. Treatment therefore, should begin within 2-4 hours for those in this age bracket. If diarrhoea is thought to be caused by prescription or non-prescription medications, discontinue their use immediately and contact your doctor. Diarrhoea caused by food allergy will subside within 4-6 hours. In the case of a persisting acute diarrhoea, whether infectious or not, a treatment must be considered, including the precautions to prevent the dehydration of the patient at the first place. Before discussing about the drugs used in the treatment of diarrhoea, the

following section will then give a short word concerning dehydration and its management during acute diarrhoea.

2.1.5 A word about dehydration

Diarrhoea occurs with fluid and electrolyte loss, due to excessive passage of watery diarrhoeal stools. Fluid and electrolyte loss can be particularly dangerous to new-borns and the elderly, who are already at higher risk for developing dehydration. Dehydration and electrolyte unbalance are the major causes of death where diarrhoea is implicated (NIDDK: National Digestive Diseases information Clearinghouse, 2001)

Symptoms, which may indicate dehydration, include:

Dry mouth.

Excessive thirst.

Less frequent urination.

Dry skin.

Light-headedness or fainting are signs of dehydration that warrant patient to be sent to the emergency room for immediate treatment.

Drinking electrolyte solutions (sports drinks), broths, and fruit juice can prevent dehydration during periods of illness.

The ORS is currently the most used solution for the treatment of diarrhoeal dehydration (Rabbani, 2000). It provides the patient with water, electrolytes, and energies, which are being lost because of diarrhoea. The amount of fluid required for oral rehydration depends on the severity of dehydration.

The solution should be taken in a small quantity at a time, but frequently (NIDDK: National Digestive Diseases information Clearinghouse, 2001)

2.1.6 *Research for better ORS*

There is no doubt about oral rehydration solution's efficiency to combat the ill effects due to diarrhoea. However, oral rehydration solution does not reduce the volume or duration of diarrhoea. On the contrary, diarrhoea may even increase (Rabbani, 2000). This reality has led to continuous studies for better ORS, in replacement of the glucose- based oral solution. For example, an oral rehydration solution modified by addition of amylase-resistant maize starch that is poorly digested in the small intestine, has been shown to be efficient in reducing the amount of diarrhoea and shorten the duration of illness in patients with cholera (Rabbani, 2000). The mechanism involved to this effect is that the indigestible starch moves through the small intestine without being absorbed and enters the colon, where it is metabolised by colonic bacteria into short-chain fatty acids. In the colon, short-chain fatty acids facilitate the absorption of water and salts, and provide an added source of energy. They also increase the synthesis of proteins, and improve the use of oxygen by the colonic mucosa (Rabbani, 2000). Other types of starches have also shown success in reducing diarrhoea. They include the poorly absorbed starch from rice, and starch in green bananas. A rice-based oral rehydration solution was found to be as effective as the glucose-based oral rehydration in the treatment of diarrhoea. Moreover, it tastes better and provides more

calories than the glucose-based oral rehydration solution. Similar results were also obtained using wheat flour and maize starches (Rabbani, 2000).

Diet of clear liquids must be started immediately upon the onset of symptoms of diarrhoea in order to prevent the dehydration of the patient. Diluted fruit juices, sports drinks, broth and teas help to keep dehydration at bay and provide the body with much needed electrolytes. During diarrhoea outbreaks, it's best to avoid dairy products, which tend to make diarrhoea worse. This includes milk, butter, creams, and eggs (NIDDK: National Digestive Diseases information Clearinghouse, 2001)

If diarrhoea is persistent or life altering, medications can be used to alleviate symptoms temporarily. One must distinguish between non-infectious diarrhoea and the infectious one. The last is treated with appropriate antibiotic drugs to kill the causal microorganism in the gastrointestinal tract, and it is not the concern of this study.

The pharmacological model used in this study for the evaluation of the antidiarrhoeal activity of plant materials was based on castor oil-induced diarrhoea in mice. The mechanism of action of the drugs that are effective against this type of diarrhoea does not involve any anti-microbial activity. Let us briefly elucidate how castor oil induces diarrhoea in mice before considering a list of commonly used non-antibiotic anti-diarrhoeal drugs and their mechanism of action.

2.1.7 Mechanism of action of castor oil as diarrhoea inducer

Castor oil induces diarrhoea through its metabolite, ricinoleic acid. The oil is hydrolysed in the upper intestine to ricinoleic acid, which has irritant properties for the mucus of the gastrointestinal tract. Ricinoleic acid increases the intestinal motility, and thereby increases the passage of water and salts, which leads to diarrhoea. The onset of action is prompt and continues until the compound (ricinoleic acid) is excreted via colon. So, the castor oil-induced diarrhoea is due to a mechanical effect, meaning that it follows an increase of the peristalsis of the GIT (Katzung, 1995) Therefore, drugs are used decreased peristalsis, an anti- motility effect on. The following is a selected list of some commonly used non-antibiotic antidiarrhoeal drugs, and their mechanism of actions.

2.1.8 Ant-diarrhoeal drugs and their mechanisms of action

There are a wide variety of anti-diarrhoeal medicines on the market. However, it is advised to stay away from over-the-counter medicines unless diarrhoea persists for about 3 days more. The most common anti-biotic antidiarrhoeal drugs include:

Bismuth

Bismuth subsalicylates (such as those found in Pepto-Bismol) are one of the safest ways to treat the symptoms of diarrhoea. Treatments containing

bismuth will help control cramping, pain, loose stools, gastrointestinal toxins, and abdominal contractions. Bismuth is safe for both children and adults.

Mechanism of action: bismuth subsalicylate appears to work by binding selectively to an ulcer, coating it and protecting it from acid and pepsin. Other postulated mechanisms of action include inhibition of pepsin activity, stimulation of mucous production, and increased prostaglandin synthesis. It may also have some anti-microbial activity against *H pylori* (NIDDK: National Digestive Diseases information Clearinghouse, 2001; Katzung, 1995).

Kaopectate

Kaopectate is a suspension formula made from absorbent clay.

Mechanism of actin: kaopectate works by absorbing compounds from solution, presumably binding potential intestinal toxins, which cause diarrhoea by irritating the gastro- intestinal tract (Katzung, 1995).

Pectin

The pectin found in apples is an age-old cure for diarrhoea. Pectin can be purchased in capsule form, or found in applesauce. Similar to kaopectate, pectin works by the mean of its ability to adsorb compounds from solution, presumably binding potential intestinal toxins.

Loperamide

Loperamide is found in many over-the-counter anti-diarrhoea medicines, including Imodium, Kaopectate II, Maalox Anti-Diarrhoeal, and Pepe Diarrhoea Control. Loperamide is an opioid derivative of phenylpiperidin

Mechanism of action: loperamide works by slowing down the movements of the intestines. Like other opioids, loperamide acts through opioid receptors which are found in high density in the gastrointestinal tract. Their constipation effects are mediated through an action of local enteric nervous system as well as the CNS. In the stomach, motility may be decreased but tone may increase, particularly in the central portion; gastric secretion of hydrochloric acid is decreased. The small resting tone is increased, with periodic spasms, but the amplitude of nonpropulsive contractions is markedly decreased. In the large intestine, propulsive peristaltic waves are diminished and tone is increased. This combination of effects delays passage of the faecal mass and allow s increased water absorption that leads to constipation or anti-diarrhoeal effects (Katzung,1995; Williamson at el., 1998)

Calcium polycarbophil

Calcium polycarbophil has been put to the test recently as a new method of treating diarrhoea and its symptoms. Best used by those suffering acute diarrhoea or irritable bowel syndrome, calcium polycarbophil works by equalising the water balance of the intestines (NIDDK: National Digestive Diseases information Clearinghouse, 2001).

2.1.8 Anti-diarrhoeal herbal medicines

It must be noted that the exact mechanisms of action of most of herbal remedies of self-medication category are not all well known. Very often, herbal drug's actions are due to a combination of compounds that are hardly separable. Moreover, the knowledge of the therapeutic properties of most of traditional herbal medicines is empirical. Only few scientific data that explain therapeutics effects of these herbal medicines are available. The list of anti-diarrhoeal herbal medicines includes:

Berries

The leaves and fruit of blueberries and bilberries help to naturally stop diarrhoea. Consuming small amounts of commercial fruit syrups and jellies is an easy way to add the benefits of berries to the diet. Berries seeds are not to be eaten, they worsen diarrhoea (Rabbani, 2000)

Chamomile

Chamomile works well with adults suffering from mild to moderate diarrhoea. Chamomile is a natural pain reliever, which help to rid of the body cramps and inflammation. Chamomile is available in tea, capsule, and liquid form (NIDDK: National Digestive Diseases information Clearinghouse, 2001)

Root bark

Perhaps the strongest diarrhoea treatment is found in the root bark of the blackberry plant. Years ago, a blackberry elixir was carried as a miracle

cure for diarrhoea. Blackberry root bark is available in tincture form at any major health food store (Take 1teaspoon of mixture in water 2-4hourly).

Vinegar

Vinegar has been in use since the time of ours grandmothers years ago, and many still swear by the vinegar cure. The dose is 1 tablespoon of vinegar each hour or until diarrhoea subsides (NIDDK: National Digestive Diseases information Clearinghouse, 2001).

Green bananas

Green bananas work to decrease intestinal porousness. It is preferably recommended when it comes to treating chronic diarrhoea in children and adults. Green bananas anti-diarrhoeal action is due to its poorly digestible starch, which moves through the small intestine without being absorbed and reach the colon. Eat 1 green banana every 2-4 hours or until diarrhoeal symptoms subside (Rabbani, 2000; NIDDK: National Digestive Diseases information Clearinghouse, 2001).

Carob

One-tablespoon of carob powder will help to soothe and calm irritated intestines. The taste leaves a little to be desired, thus one may want to mix it with a fair amount of plain yogurt or applesauce (NIDDK: National Digestive Diseases information Clearinghouse, 2001).

Catnip

Catnip tea is a great way to reduce spasmodic intestines. Drink 1 cup every three hours (Shale at el.,1999).

2.1.10 The Western Cape antidiarrhoeal herbal medicines

In Western Cape, as in most of developing countries, traditional medicine mainly relies on the use of herbal remedies, *P. triste*, *E. rhinocerotis*, *and O. euro. africana* are some of the most used plants to treat diarrhoeal disease in the Western Cape. The aim of this study was to validate the claims by traditional medicine practitioner concerning the antidiarrhoeal activity these plants. The choice of these plants for scientific investigation was justified by their widespread popularity in the Western Cape traditional medicine, their availability, and more important, the lack of scientific data supporting their therapeutic properties. A short literature survey about the three medicinal plants, *P. triste, E. rhinocerotis,* and *O. euro africana* is given in the following pages.

2.2 ABOUT THE MEDICINAL PLANTS: *Pelargonium triste*, *Elytropappus rhinocerotis*, and *Olea europaea africana*

2.2.1 Pelargonium triste

The plant is also known in Afrikaans as “Kaneeltjie” or “Rooiwortel”. The term Tristis (Latin), refers to the dull colour of the flowers. It is a geophyte with a large subteranean tuber and tuberous roots. The main stem is usually short and succulent. Before dormancy, all the epiterranean parts of the plants die off. The radical leaves resemble those of a carrot. They vary

considerably in hairiness and structure the segments decurrent, toothed or lacinate. The leaf blades are oblong ovate in outline, measuring approximately 10-45 cm long and 4-15 cm broad. Leaves on the flowering stems are usually much smaller than those on the main stem. Large cordate stipules are found at the base of the hairy petioles, and typical star-like and night-scented inflorescence of the Poliactum. Pseudo-umbels with 6-20 flowers are borne on very long hairy peduncles. The plant flowers from August to February. The petals, of a dull coloured yellowish-green to brownish-purple hue, are edged with a lighter margin. Seven fertile stamens are present. The root of the plant is edible.

Medicinal uses

Infusions of the fleshy tubers were used for diarrhoea and dysentery, as well as an anthelmintic. Several other species have similar fleshy underground structures that are also used as antidiarrhoeal (Van der Walt, 1977).

Distribution

The plant occurs naturally in a strip running parallel to the Northwest coast of Western Cape, South Africa, from Steinkopf upwards. It usually grows in sandy soil. A subterranean network often gives rise to a population of separate plants (University of the Western Cape and South African Medical Research Council, 2000).

2.2.2 *Elytropappus rhinocerotis*

The plant belongs to the family of Asterceae. It is also called "Stoebe rhinocerotis L.f.", or "Renosterbos, or Resterbostoppe" in Afrikaans. This is indigenous to the Cape Province. *Elytropappus rhinocerotis* is a many-branched grey to grey-green aromatic shrub of 0.6-2.5 m in height with young densely woolly stems. The minute, greyish-green leaves are tightly grouped on the thin stems. The tiny flower heads are inconspicuous, yellow and tubular, with a single floret in each. It flowers between March and September. The fruit is an achene with prominent longitudinal rib (University of the Western Cape and South African Medical Research Council, 2000).

Medicinal uses

The yellow tips of the branches are used. Infusions of young branches, in a brandy or wine, are traditionally used for indigestion, dyspepsia, ulcers, and stomach cancer. It was reported to be a popular remedy during the 1918 influenza epidemic because it stimulates perspiration. Infusions or tinctures are traditionally used; taken in small amounts three times a day (University of the Western Cape and South African Medical Research Council, 2000)

Distribution

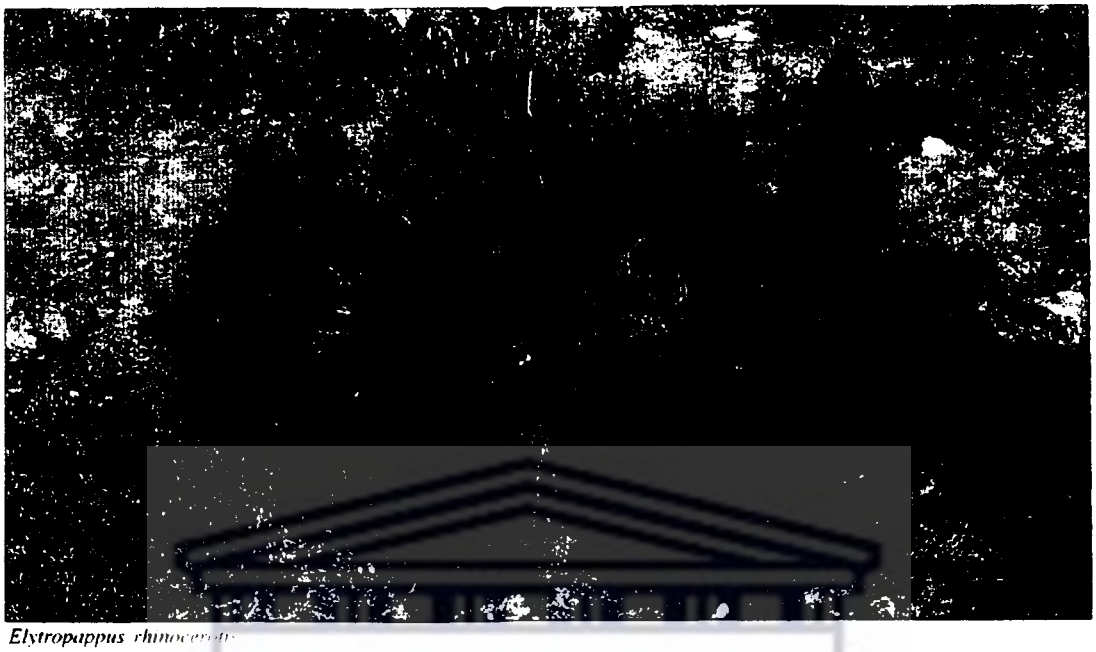
Common on dry clay flats and slopes throughout the Western and Eastern Cape Provinces, up to Namaqualand in South Africa, it is capable of

forming pure stands covering a large area, renosterveld (University of the Western Cape and South African Medical Research Council, 2000)

2.2.3 Olea europaea africana

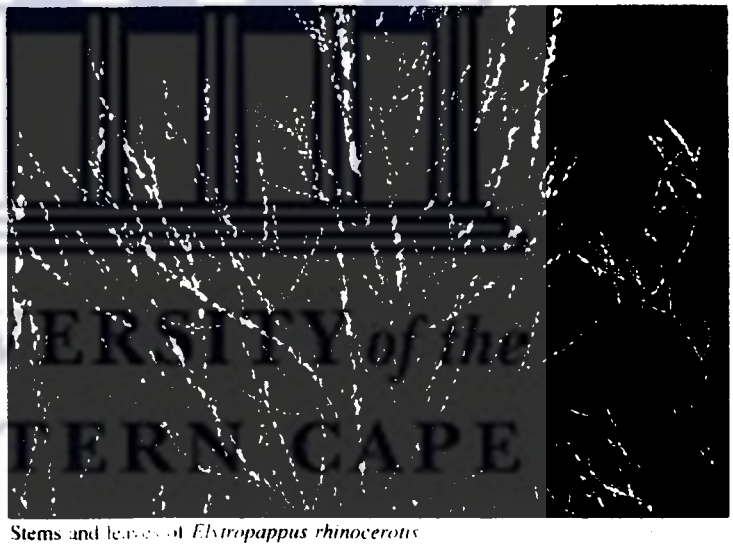
It is also commonly called "*Olea africana Mill*". *Olea europaea*, the origin of the cultivated olive, is very widespread in Mediterranean countries, Africa, the Arabian Peninsula, the Indian subcontinent and Asia. Several subspecies are recognised; one of which is the small-fruited subspecies africana (formerly Olea africana). This is a tree of 3-15m in height, which may assume bush habit if stunted. Its leaves are simple, opposite, lanceolate to elliptic, 20-90mm × 7-15mm, and grey-green to shiny green on upper surface. It is greyish or yellow-gold on lower surface due to the presence of fine dense scales, margins reflexed and lamina curling downwards. Flowers appear between October and March. They are fragrant, 6-10mm long, cream to white, borne in lax axillary panicles, whitish spots. They become black to purple when ripe.

Figure 1: *Pelargonium triste* plant



Elytropappus rhinocerotis

Tincture of *Elytropappus rhinocerotis*



Stems and leaves of *Elytropappus rhinocerotis*

Figure 2: *Elytropappus rhinocerotis* plant in its natural habitat

Figure 3 : *Olea europaea africana* plant in its natural habitat

Medicinal uses

Dried leaves are mainly used, but sometimes the roots or stem bark are used as well. The dosage forms commonly used include infusions or decoctions, taken orally or applied locally. The infusion is traditionally used in the Montagu district (Western Cape province, South Africa), for throat, kidney problems, and backache. Leaf infusions are used elsewhere to treat eye infections or as a gargle to relieve sore throat. These also may be taken orally as a remedy for colic, urinary tract infections or to lower the blood pressure (University of the Western Cape and South African Medical Research Council, 2000).

Pharmacological activity

While little work have been carried out concerning pharmacological investigation of the South African populations of *O. euro. africana*, some information can be found from studies elsewhere. For example, hot water extracts of *Olea argentinean's* leaf, at a concentration of 62.5 mg/ ml, were found to be inactive against *Staphylococcus aureus*, *Aspergillus niger* and *Escherichia coli* (agar plate method), whereas 95% alcohol extracts were found to be active against microbes including *Mycobacterium*, using broth culture methods (University of the Western Cape and South African Medical Research Council, 2000). Antiarrhythmic activity of 95% ethanol, glycerin and ethanol: glycerin (50:50) extracts of *O. europaea africana's* leaf and shoot have been demonstrated in the rat at doses of 25mg/kg, following aconite-induced arrhythmia. Other studies have also demonstrated other pharmacological activities including anti-spasmodic activity, diuretic,

hypoglycaemic activity, and anti-inflammatory effects (University of the Western Cape and South African Medical Research Council, 2000).

Distribution

The wild olive grows in a variety of habitats, from forest and riverside bush to open grassfield, stony flats, mountain tops and rocky ledges throughout Southern Africa and northwards through East tropical Africa into Eritrea (University of the Western Cape and South African Medical Research Council, 2000).

2.3 A WORD ABOUT THE RISING INTEREST IN PHYTOPHARMACY

2.3.1 Historical

Throughout history, humans have looked to nature to provide them with medicines. The use of plants with medicinal properties forms the basis of various traditional medicinal systems. This practice has existed for thousands of years, and still play major as care provider nowadays (Bullen at el., 1999). The first records, which date back to Mesopotamia (2600 BC) documented the use of Cedar, Cypress, Liquorice and Myrrh oils to treat ailments ranging from coughs and colds to parasitic infections and inflammation (Bullen, 2000). Egyptian pharmaceutical records have documented some 700 drugs derived mostly from plant, including gargles, snuffs, poultices, infusions, pills and ointments. But, while recorded history of medicinal plants is largely attributed to the meticulous records from Egypt, China, India, Greece and the Arab world, traditional African and

South American cultures have also relied heavily on nature for medicines (Bullen, 2000).

2.3.2 Phytopharmacy and the Modern Pharmaceutical Industry

Modern pharmaceutical industry has a long history of looking to nature to inform its researches. In 1928, Alexander Fleming's laboratory in London discovered the active ingredient, penicillin, which ultimately became one of the most effective drug for the treatment of pneumonia, syphilis, wound and childbirth infections. Almost a century earlier, in 1820, French pharmacists Caventou and Pelletier isolated the anti-malarial drug quinine, from a cinchona bark, and which has long been used by indigenous people to treat fever. Today quinine is the basis of many anti-malarial drugs. Chloroquine and mefloquine are analogues of quinine. During the last few decades, a remarkable resurgence of interest in phytotherapy and phytopharmacy has taken place. Herbal medicine is currently enjoying a revival even in the West where previously it had little consideration. The traditional Chinese, Ayurvedic and Unani systems of medicine for instance, are spreading throughout the world with increasing popularity (Williamson at el.,1998). Many natural products have become standard drugs of modern medicine, that many laymen and even some members of the medical profession are unaware of their plant origin. Theophylline, caffeine, digoxin, hyosine (scopolamine), ergometrine, ephedrine, pilocarpine, vicristine and vinblastine are but a few examples in this category of drugs. Plant compounds have also served as templates for the semi-synthesis of *a*

number of drugs. The local anaesthetic, lignocaine, was derived from cocaine; neuromuscular blockers from tubocurarine; and bromocriptine from ergot alkaloids (Williamson at el., 1998).

What may be the factors that have contributed to the rising of interest for phytotherapy and phytopharmarcy in these last decades? We will try to answer to this question.

2.3.3. The reasons for the resurgence of interest in phytopharmacy

According to Williamson and colleagues (1998), the following factors may justify the remarkable resurgence of interest in phytotherapy and phytopharmacy these last decades:

* The expectation from the public in developing countries that their drug problems can be alleviated through a sensible scientific exploitation of medicinal plants from their home's flora and fauna, some of which have been used traditionally for generations.

* The fact that herbal medicine has quite often supplied efficient treatment for some conditions for which conventional medicine run short of remedies or had little to offer support the above mentioned expectation. This is the case for instance with liver damage, where lignans from Silybum marianum can prevent fatalities caused by the "death cap mushroom", and hepatitis, and immune stimulants such as the polysaccharides from the coneflower, Echinacea, are used to treat viral infections (Williamson at el., 1998).

* The world- wide "green revolution", which is reflected in the belief that herbal remedies are safer and less damaging to the human body than synthetic drugs is also element in favour of phytotherapy.

• Finally, the fact that many important drugs in use today were derived from plants or from a molecule of plant origin also justifies the strong return of interest in phytotherapy and phytopharmacy. For example digoxin, quinine, warfarin, and reserpine are all but drugs of plant origin. Furthermore some plants have also yielded molecules which have considerably helped as tools in the characterisation of enzymes and classification of receptor systems: muscarine, nicotine' physostigmine, and tubocurarine are important examples. Not all plants in the forest are of interest for phytopharmacy researches. What are then the criteria involved in the selection of plants for phytopharmacy researches? This question will be answered in the following lines.

2.4 HOW TO SELECT PLANTS FOR PHARMACOLOGICAL ACTIVITY INVESTIGATION

2.4.1 Criteria for selection of plants

The following criteria are commonly used in the selection of plants for pharmacological activity investigation:

i. Usually, scientists select plant species for phytochemical and/or Biological screening following claims and uses by traditional medicine practitioners in the treatment of diverse ailments.

ii The need to address a specific community's demand is another criterion which may determine the choice of plants for phytochemical and biological studies. Such need, may be the need to provide drugs that would be more culturally acceptable to the public or the need to find cheaper drug and so on. In fact, there is actually a growing expectation that cheaper and efficient remedies can be found through exploitation of our neighbouring forest and sea.

iii The cost involved in the collection and the growing (replacement) of plant species plays also a determinant role when selecting plants for study purposes. In order to obtain a cheap drug all the production costs, for example must be kept low, including cost for plant replacement.

iv. The selection of medicinal plant species can also be purely based on chemical composition of plants. A laboratory may decide, because of available facilities to extract a certain class of compounds, such as alkaloids, for investigation. Then plants which are supposed to contain alkaloids according to chemotaxonomic information will be selected, no matter whether they are used in traditional medicine or not, and screened on as wide a range of models as possible.

v. It has also become possible, on the basis of computerised databanks, to predict that one species of plant is more likely to yield an activity of interest than other species, and the choice can the follow (Williamsons at el., 1998).

vi Generally a combination of two or more of the above criteria is used instead of a single criteria (King's College, 1998).

2.4.2 How to handle a plant materials for phytopharmacy research

The preparation of plant material for scientific investigation starts with a proper botanical identification of the plant that is about to be investigated. An acknowledged authority must authenticate the botanical identity of the plant under investigation. Many mistakes over plant's identity have occurred in the past that it is essential to authenticate the material whenever reporting new substances from plants or even known substances from new plant sources. It is good practice therefore, in phytochemical research, to deposit a voucher specimen of the plant examined in a recognised herbarium for referential purposes.

Plant collection

Depending on the usefulness of the parts of the plants (leaves, flowers, stems, fruits or roots), good plant collection may impose special conditions with regard to the weather, time of collection (seasonal or diurnal), age (young or adult), and health condition of the plant, the method of collection and so on (King's College Dept. of Pharmacy, 1998).

Storage

A number of elements must be taken into account in order to insure better preservation of plant material in storage. Storage of damp plant material for example, will lead to spoilage by fungi, bacteria or fermentation. In this case, drying is advised. It is the commonest method of preservation and reduces the weight of material for packing and transport. But there also,

precaution needs to be taken if denaturing of compounds is likely to occur. Fresh ginger, for example, contains gingerols, which are degraded to the more pungent shogaols during the dehydration process. It must be noted in general, that the method of drying, for example, in sunlight, artificial heat or vacuum and concomitant processes can influence the quality of the active substance (King's College Dept. of Pharmacy, 1998)

Drying with heat will dissipate essential oils and destroy thermolabile constituents. Deep freeze-drying would be safer in this case. Also, alcohol can be used as a preservative and/or solvent of plant material, bearing in mind that it is bulky and inflammable. Higher temperatures can also cause gelatinisation of starch, coagulation of proteins and decomposition of important constituents.

It is useful some times to store plant materials in dried powdered form, which requires less space and allows storage in tightly closed containers such as bottle. Collected plant parts will then be sliced into smaller size particles and then ground into powder form. However, there is no perfect method that would be applicable to all. So, advantages and disadvantages of each method of storage must be evaluated to determine what will be appropriate for each case (King's College Dept of Pharmacy, 1998). The type of research project to be conducted and the appropriate methods to be followed are also determinant factors in the choice of a mode of preservation of material plants.

So, different studies use different mode of preservation of plant raw materials, also depending to different models of investigation the research

project intends to follow. For example, there are several models for the evaluation of the antidiarrhoeal activity of a plant material that can be utilised. In the following lines, some of commonest models used for pharmacological evaluation of antidiarrhoeal activity of drugs will be discussed, including the in vivo model based on castor oil-induced diarrhoea of Williamson (1998), whose principle is applied in the present study.

2.5 MODELS FOR PHARMACOLOGICAL EVALUATION OF PLANT MATERIALS FOR ANTIDIARRHOEAL ACTIVITIES

Introduction

Preparations used as antispasmodics (also called carminatives or spasmolytics) in the gastro-intestinal tract may also be used in other disorders such diarrhoea, since they are utilising a common smooth muscle relaxant effect. Spasmolytics compounds such as hyoscine, which has powerful effects on peristalsis, are particularly useful for treating not only colic and indigestion but also diarrhoea, dysmenorrhoea and as anti-emetic (Williamson at el., 1998). It exerts its effects via its anticholinergic activity. Examples of plant-derived antispasmodics include some tropane alkaloids (atropine, hyoscine or scopolamine); opium alkaloids (papaverine, codeine, and loperamide); essential oils (chamomile); and flavonoids. Testing for antispasmodic activity would then mean testing antidiarrhoeal activity.

2.5.1 In vitro methods

In vitro testing is usually carried out by observing any direct effects on isolated intestine preparations in organ bath, e.g. guinea-pig ileum, rat duodenum or rabbit jejunum. Spasms then induced by a number of agonists, the nature of which may give a good indication of the mechanism of action.

Isolated Guinea-pig Ileum and related preparations

A segment of ileum 2-4 cm long from a freshly killed guinea-pig is suspended in an organ bath (5 – 15 ml) containing Tyrode solution (pH 7.4) at 34 – 37 °C aerated with 5% CO_2, 95% O_2, loaded with a suitable weight or tension (about 0.5g). The longitudinal contractions are recorded on a kymograph or Dynograh recorder running at a speed of about 5mm/ min.

Reagents: * Tyrode solutions:

Tyrode solution formula

Salt	Conc. (g/l)
NaCl	8.0
KCl	0.2
$Ca\ Cl_2$	1.8
$NaHCO_3$	1.0
$MgCl_2.6H_2O$	2.0
$NaHPO_2$. 2H2O	0.062
Glucose	1.0

Agonists (spasmogens): Cetylcholine, Barium chloride, histamine, Prostaglandin, and calcium chloride.

Antagonists (Antispasmodics, spamolytic):

Papaverine (as a positive control)

Test plant extract: dissolved in alcohol, Tyrode solution or emulsified in Tween 80 if necessary (Williamson at el., 1998).

2.5.2 In vivo methods

Gastro-intestinal Transit of Charcoal Meal Method:

This method involves measuring the transit time of charcoal meal, which is visible in the gastro-intestinal tract of mice.

Protocol: The animals are fasted 24 h prior to the experiment, pre-treated with test plant extract and 15 min later given orally the charcoal meal consisting of 0.4ml of an aqueous suspension of charcoal in 55 gum acacia. The animals will be killed by ether inhalation 20 min after the meal , and the intestines and stomach removed. The pylorus is attached to a glass rod and the intestine suspended for 20 sec with a weight of 3g attached to the ileocaecal junction, to straighten it out. The mean distance travelled by the charcoal can then be measured and compared with the control group expressing the results as a percentage of the total length of intestine (Williamson at el., 1998).

Castor oil-induced diarrhoea method

Principle: Administration of castor oil induces diarrhoea in mice (90% of animals) within 4h. Pre-treatment with an antidiarrhoeal plant extract will reduce the number of animals exhibiting diarrhoea and also reduce the incidence. Protocol: Mice are divided into two groups of 30 animals each; the first group receiving a suitable placebo, in a similar dose and volume to the test group receiving plant extract. 30 min later both groups are treated with castor oil (o.5ml/animal, p.o.) and each mouse kept for observation

under glass funnel on tissue paper. Onset of diarrhoea and the number of such episodes are noted for each animal, for a total of 6h. Statistic comparison of mean values of total diarrhoeal episodes in control and test groups is made using Student's t-test (Williamson at el., 1998).

CHAPTER 3

MATERIALS AND METHODS

3.1. Plant materials

3.1.1 Collection and authentication of plants

The selection, collection, and identification of medicinal plants, *P. triste*, *E. rhinocerotis,* and *O. euro. africana* resulted from a combination of methods including data from the literature, and communication with traditional medicine practitioners. These plant species were all identified and collected in the areas of Cape Town. The first plant, *P. triste* was collected from "Farm Klein Melbosch" 3318 CB Cape Town, Western Cape, and verified by Franz Weitz, a taxonomist at the University of the Western cape. The voucher specimen (TRAD 142) was deposited in the herbarium of the Department of Botany University of the Western Cape. The second medicinal plant, *E. rhinocerotis* was collected at the " Herb Garden", Montagu Museum, 3320 cc Montagu, South Africa, and verified by the Botanist of the Garden. The voucher specimen (TRAD173) can be found at Herb Garden, Montagu Museum. *Olea euro. africana* was collected and validated at "Karroo Botanic Garden", (NBI), Worcester 3319 CB, by the curator Botanist at the garden. The specimen (TRAD 201) of the plant was deposited at the garden's Herbarium.

3.1.2 Preparation of aqueous extracts

The active parts of the plants were cut off, sliced, and then dried in an oven at 30°C. These include fleshy tubers of *P. triste*, young branches and leaves of *E. rhinocerotis*, and leaves and stems of *O. euro.* africana. Dried parts were finally ground into a fine powder using a "Moulinex" coffee grinder, and then sieved through, using 850 μm sieve- mesh size. Approximately 20g of each plant material dried powder was infused and constantly shaken up in 1 litre of boiled distilled water in a 2-litre Erlenmeyer container. The infusion was allowed to cool over 24 hours, and then filtered through cotton wool in a large funnel.

The resultant filtrate solution was then frozen at a temperature of -80 °C in a freezer (Lozone Cfc Free Freezer, Model U85360, New Brunswich Scientific, USA) for 24 hours, and then freeze-dried using Savant Freeze Drying System consisting of "Refrigerated vapour trap (Speed Vac. SC110 SAVANT, Farmmingole, NY, USA), and a freeze drying chamber (Model FDC 906, and Digital Vacuum Gaugle, Model DVG 50).

3.1.3 Preparation of drugs solutions

Different quantities of freeze dried extract of each medicinal plant were weighed, dissolved and made up to the appropriate volumes with normal saline. Fresh solutions were prepared on the days of the experiments.

3.2 Drugs and chemicals

Castor oil, prepared and bottled by GR. Pharmaceuticals (Pty) Ltd., Atlantis 7349 South Africa, batch number 1263, and Loperamide (4-[p-chlorophenyl]-4-hydroxy-N,N-dimethyl-∝,∝-diphenyl-1-piperidine-butyramine) hydrochloride, Sigma Chemical Co. were used. Castor oil was warmed to a temperature of 30- 35 °C in a warm water bath before and during utilisation. Loperamide was dissolved in 10% ethanol (prepared from ethanol 99%, Merck (Pty) Ltd.), and made up to the appropriate volume with distilled water. All the drugs were administrated orally to the animals.

3.3 Animals

Male albino mice bred in the animal house of the Department of Pharmacology, University of the Western Cape, Bellville, South Africa, weighing 18-25 g were used. The animals were kept in groups of 10-12 per cage with free access to food and tap water. Eight mice per test group. Each animal was used for one experiment only.

3.3 Assessment of antidiarrhoeal activity

A modified method of Williamson at el.(1998) was used to assess the antidiarrhoeal activity of the plant materials.

The principle of this method is that the administration of castor oil induces diarrhoea in mice (90% of animals) within 4 hours of administration. Pre-treatment with an antidiarrhoeal plant extract will reduce the number of animals exhibiting diarrhoea and also reduce the incidence.

No starving male albino mice (weighing 18-25 g) were divided into test groups containing eight animals each. Each test group was pre-treated with a dose (ranging from low to high dose) of plant extract (according to animal's body weight) for 15 minutes after which 0.7ml of castor oil was given orally. Each mouse was then kept for observation in a large beaker (5000ml) on a white tissue paper (filter paper). The onset of diarrhoeal activity, the number of diarrhoeal episodes, and the total stool mass were noted at hourly intervals for a period of 5 hours after the administration of castor oil. In order to determine the total stool mass, the animal's bedding (the white tissue paper) was weighed before and after diarrhoea, and also renewed at the same interval of time (1hr).

The experiment was also repeated for all control groups, including the group pre- treated with 0.25ml normal saline solution orally, group pre-treated with 0.25ml ethanol 10%, group pre-treated with loperamide, and the control group that received 0.7 ml castor oil (Williamson at el., 1998).

3.4 HPLC analysis

i Chromatographic system

The chromatographic system used in the experiments was a Beckman HPLC system consisting of one double pump Programmable Solvent Module, model 126; one Diode Array detector Module model 168; a Samsung computer 386 with management System Gold (GoldV601) software supplied by Beckam; and a Column, C18 Bondapak 5 μm dimensions (250 × 4.6 mm).

ii Chromatographic conditions

The mobile phase of the chromatographic system included solvent A: 1% CH3COOH; solvent B: MeOH, a mode gradient, and a flow rate of 1ml/min. The injection volume was 20 μl. The UV detector was at 270 nm. The reference standard: Rutin 2.5 g was dissolved in 100ml MeOH.

iii Sample preparation

250mg of freeze-dried extract were dissolved in 5ml of distilled water. The HPLC operating conditions were programmed as following: At 0 min, solvent B: 20%; at 5 min, solvent B: 40%: at 15 min, solvent B: 60%; at 20 min, solvent B: 80%; and at 27 min, solvent B: 20%. The run time period was 30 minutes.

3.6 Phytochemical analysis

Plant extracts were screened for various constituents using standard screening tests and conventional protocols

i. Tests for alkaloids

200mg freeze- dried powder of aqueous extract of each medicinal plant was dissolved in 10% hydrochloride acid in ethanol. To one ml of the obtained solution were added three drops of "Dragendorffs' Reagent" (potassium bismuth iodide solution). An orange-red precipitate would indicate the presence of alkaloids. The same test was repeated using "Mayers' Reagent" (potassium mercuric iodide solution) and a pale precipitate would indicate the presence of alkaloids. No precipitate occurred (*British Pharmacopoeia*, 1980).

ii General chemical test for glycosides.

It must be noted that there is no general test for glycosides (as they are heterogeneous group), apart from the presence of sugar after hydrolysis.

The principle is that glycosides are soluble in water, and acid hydrolyses glycosides to reducing sugars and aglycones.

Protocol: 0.5g of each medicinal plant material in powdered form was boiled in 5ml of distilled water. An equal volume (2ml) of Fehling A and B solution was added to the solutions, and gently heated on a water bath. A

red brown precipitate would indicate the presence of reducing sugars. (Farnsworth, 1966).

iii Tests for tannins

0,1g of freeze dried powder of aqueous extract of each medicinal plant material was dissolved in 5ml of distillate water, 5 drops of 5% ferric chloride solutions were added to the obtained solutions, a blue-black precipitate indicated the presence of tannins. (Farnsworth, 1966).

iv. Tests for Saponins

Saponins are a particular form of steroids or triterpenoid glycosides, which are characterised by surfactant properties. This property was used for their detection: 0,2g of freeze dried powder of aqueous extract of each medicinal plant material was dissolved in 10 ml of distilled water, and vigorously shaken in a test- tube. A persistent froth or foam would indicate the presence of Saponins (Farnsworth, 1966).

v. Tests for Anthraquinones (Borntrager's Test, BPC)

0,1g of the powder of each medicinal plant material was shaken up with 10 ml of Ferric Chloride solution (15%) and 5 ml of hydrochloride solution 1N in a warn water-bath for about 10 minutes, and filtered and allowed to cool. The filtrate was extracted with 10 ml of carbon tetrachloride. The carbon tetrachloride layer was separated and washed with 5 ml of diluted ammonia solution. A red-pink colour (or cherry red) in the ammoniacal layer indicates

the presence of an anthraquinone. Anthraquinone-aglycone forms (which are oxidised anthracenes) give a pink (reddish) colour in presence of alkali. Reduced anthracenes can be converted to Anthraquinones with Iron (III) chloride. (Farnsworth, 1966).

3.7 Statistic analysis

All the data were expressed as the mean ± standard error of the mean (SEM) and analysed using the paired t-test; n = 8. The P values of less than 5% ($p < 0.05$), were considered to be significant.

CHAPTER 4

RESULTS AND DISCUSSION

The evaluation of the antidiarrhoeal activity of medicinal plants under this investigation involves the measurement of the effects of the plant aqueous extracts the onset of diarrhoea, the total diarrheoal stool mass and the number of animals exhibiting diarrhoea. The effects of each drug (expressed as mean ± standard error of the mean (SEM); n = 8) were compared with the effects of the control, castor oil. The data with p values of less than 5% ($p < 0.05$) were considered significant. The following discussion will then be divided into three sections related to the effects of the drugs on the onset of diarrhoea, the effects on total diarrhoeal stools mass, and the effects on the number of animals exhibiting diarrhoea.

4.1 Effect on the onset of diarrhoea

i Effects of castor oil

The administration of castor oil (0.7ml/animal, po.) to mice induced diarrhoea within 58.88 minutes period of time.

ii Effects normal saline

Mice were pre-treated with 0.25ml of normal saline solution, 15 minutes before the administration of castor oil. No significant difference was found between the onset of diarrhoea for the control group pre-treated with

normal saline solution and the group treated with castor oil only. This implies that normal saline did not affect the diarrhoea-inducing effects of castor oil in mice, nor did it affect the effects of the plant extracts on the onset of castor oil-induced diarrhoea.

iii Effects of loperamide

Loperamide was used as positive control. No diarrhoeal activity was noted in mice pre-treated with loperamide 25 mg/kg, 50 mg/kg, and 75 mg/kg, 15 minutes prior to the administration of castor oil. Loperamide is an experimental antidiarrhoeal standard reference. It is also used clinically in the treatment of diarrhoea.

iv Effects of ethanol 10%

Mice were pre-treated with 0.25ml of ethanol 10%, 15 minutes before the administration of castor oil. No significant difference was found between the onsets of diarrhoea for the group of mice pre-treated with alcohol 10% and the group treated with castor oil only. This implies that the alcohol that was used as vehicle for loperamide did not affect the effects of loperamide on the onset of castor oil-induced diarrhoea in mice.

v Effects of plant extracts

At the dose of 50 mg/kg, and 75 mg/kg, *P. triste* and *E. rhinocerotis* aqueous extracts significantly delayed the onset of castor oil-induced diarrhoea in mice. This implies that *P. triste* and *E. rhinocerotis* aqueous

extracts have inhibitory properties against castor oil-induced diarrhoea type. The fact that at the dose of 25 mg/kg *P. triste* and *E. rhinocerotis* did not significant delay the onset diarrhoea suggests that the effects of these extracts are dose related.

Olea euro africana did not significantly affect the onset of castor oil-induced diarrhoea in mice, except at the dose of 50 mg/kg. It means that the effects of *O. euro africana* on the onset of castor oil-induced- diarrhoea in mice are not dosing dependent (Table 1).

4.2 Effect on total diarrhoeal stool mass

i Effects of castor oil

The total diarrhoeal stool mass per mouse, recorded over 5-hrs period of time, after the administration of castor (0.7ml/ animal, po.) was 580 mg.

ii Effects of normal saline

There was not significant difference between the total diarrhoeal stool masses for animals pre-treated with normal saline and the animals treated with castor oil alone. This means that normal saline did not affect the effects of plant extracts on the total diarrhoeal stool mass of mice.

No diarrhoeal activity was noted for the animals pre-treated with loperamide, 15 minutes prior to the administration of castor oil.

This means that loperamide caused total blockage of castor oil-induced diarrhoea in mice.

iv Effects of ethanol 10%

There was no significant difference between the total diarrhoeal stools mass for animals pre-treated with alcohol 10% and the animals treated with castor oil alone. This implies that alcohol 10% that was used as a vehicle for loperamide did not affect the effects of loperamide on to total diarrhoeal stool's mass of animals.

V Effects of plant extracts

At the doses of 50 mg/kg and 75 mg/kg, *P. triste* and *E. rhinocerotis* significantly reduced the total diarrhoeal stools mass. But no significant difference of the total diarrhoeal stools mass was found at the dose of 25 mg/kg, when compared with the control.

This suggests that *P. triste and E. rhinocerotis* possess inhibitory effects on diarrhoeal stool mass, and these effects are dose related and/or short acting. *Olea euro africana* did not significantly reduce the total diarrhoeal stools mass of animals, except at the dose of 25 mg/kg. This implies that *O. euro africana's* inhibitory effect on diarrhoeal stools mass is not dose related (Table 1).

4.3 Effects on the number of diarrhoeal episodes

The number of the diarrhoeal episodes per animal (over a period of 5 hours after the administration of castor oil to mice) was 4.13. There was no significant difference between the numbers of diarrhoeal episodes for

animals pre-treated with normal saline and animals treated with castor oil alone. It means that normal saline did not affect the effects of plant extracts on the number of animals exhibiting castor oil-induced.

No diarrhoeal activity was noted for the mice pre-treated with loperamide. There was no significant difference between the number of animals exhibiting diarrhoea for the animals pre-treated with alcohol 10% and the animals treated with castor oil only. This implies that alcohol 10% that was used as a vehicle did not affect the effects of loperamide with regard to the number of animals exhibiting diarrhoea.

At the doses of 25, 50, and 75 mg/kg, all the plant extracts, *P. triste, E. rhinocerotis*, and *O. euro africana* significantly reduced the number of animals exhibiting diarrhoea. This implies that *P. triste, E. rhinocerotis, and O. euro africana* has significant antidiarrhoeal properties. The antidiarrhoeal effects of these medicinal plants reach their highest level within one-hour period. There is a constant diminution of the number of diarrhoeal episodes three hours after the administration of castor oil. This is not a result of the antidiarrhoeal effects of the plant extracts but a consequence of the dehydration and exhausting effects caused by 3 hours of active diarrhoea of the little mice.

Table1: Effects of plant extracts on castor oil induced diarrhoea in mice.

Treatment (Extract or agent)	Dose mg/ kg)	Average number of diarrhoeal episodes	Onset of diarrhoea. (minutes)	Average total stool mass (Mg ± m.e.s)
Castor oil	0.7 ml/ anim.	4.13 ± 0.44	58.88 ± 4.88	580 ± 60.10
P. triste	75	2.5 ± 1.1*	108.14 ± 18.8 *	240 ± 49.49 *
	50	2.63 ± 1.03 *	100.67± 12.02 *	360 ± 12 *
	25	3 ± 0.27*	72.13 ± 8.87	540 ± 53.03
E. rhinocerotis	75	2.13 ± 0.48*	79.71 ± 15.67*	350 ± 63.63*
	50	3.13 ± 0.44*	77.5 ± 11.85*	520 ± 20.11*
	25	2.76 ± 0.37*	70.78 ± 13.24	560 ± 91.92
O. euro africana	75	3.13 ± 0.58*	71.43 ± 14.87	560 ± 75.56
	50	3.38 ± 0.32*	86.62 ± 15.03 *	540 ± 60.10
	25	2.38 ± 0.46*	71.38 ± 13.07	410 ± 67.17*
Loperamide	75	0 *	0*	0*
	50	0 *	0*	0*
	25	0 *	0*	0*
Ethanol 10%v/v	0.25 ml	4.88 ± 0.29	50.86 ± 9.73	680 ± 49.50
Normal saline	0.25 ml	420 ± 0.15	60.82 ± 4.22	600 ± 20.00

*p< 0.05 versus castor oil control. Student's t-test. N =8

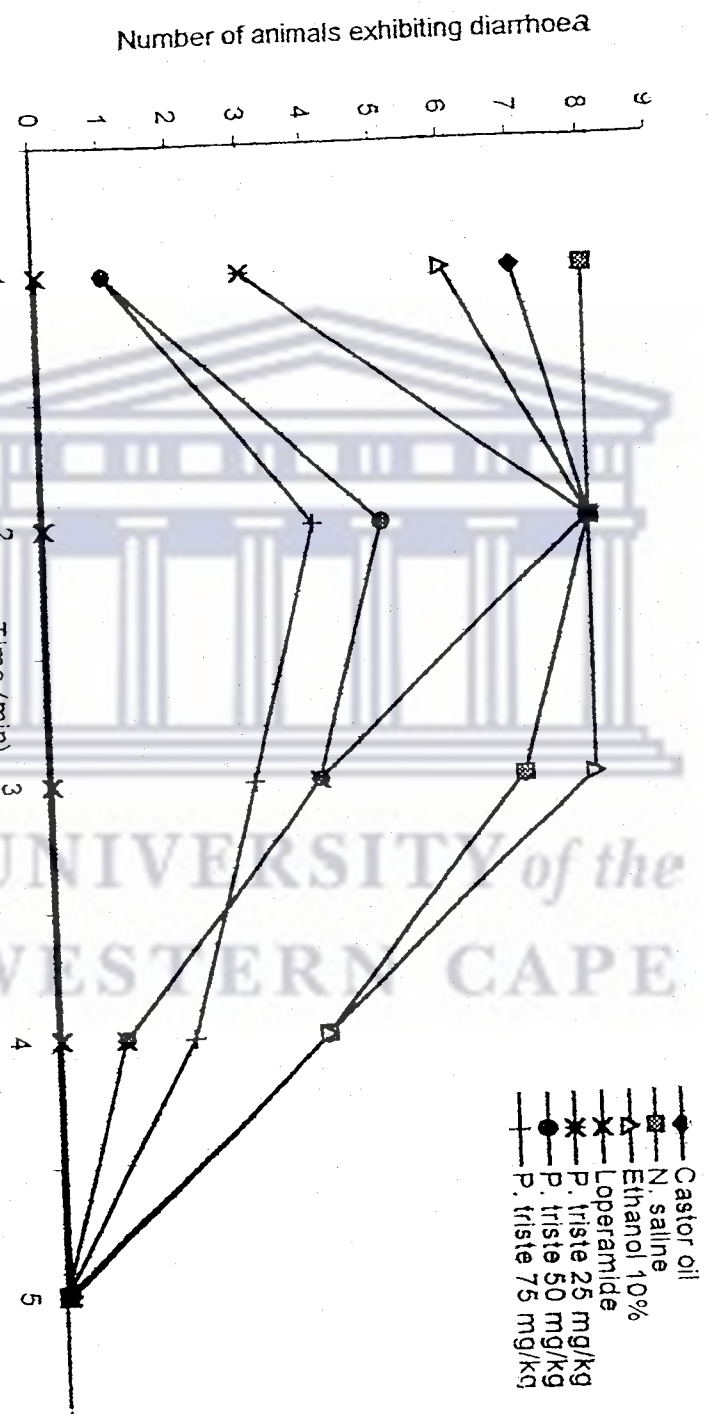


Figure 4 : Effects of *P. triste* (25mg/kg, 50 mg/kg, 75 mg/kg) on castor oil-induced diarrhoea in mice.

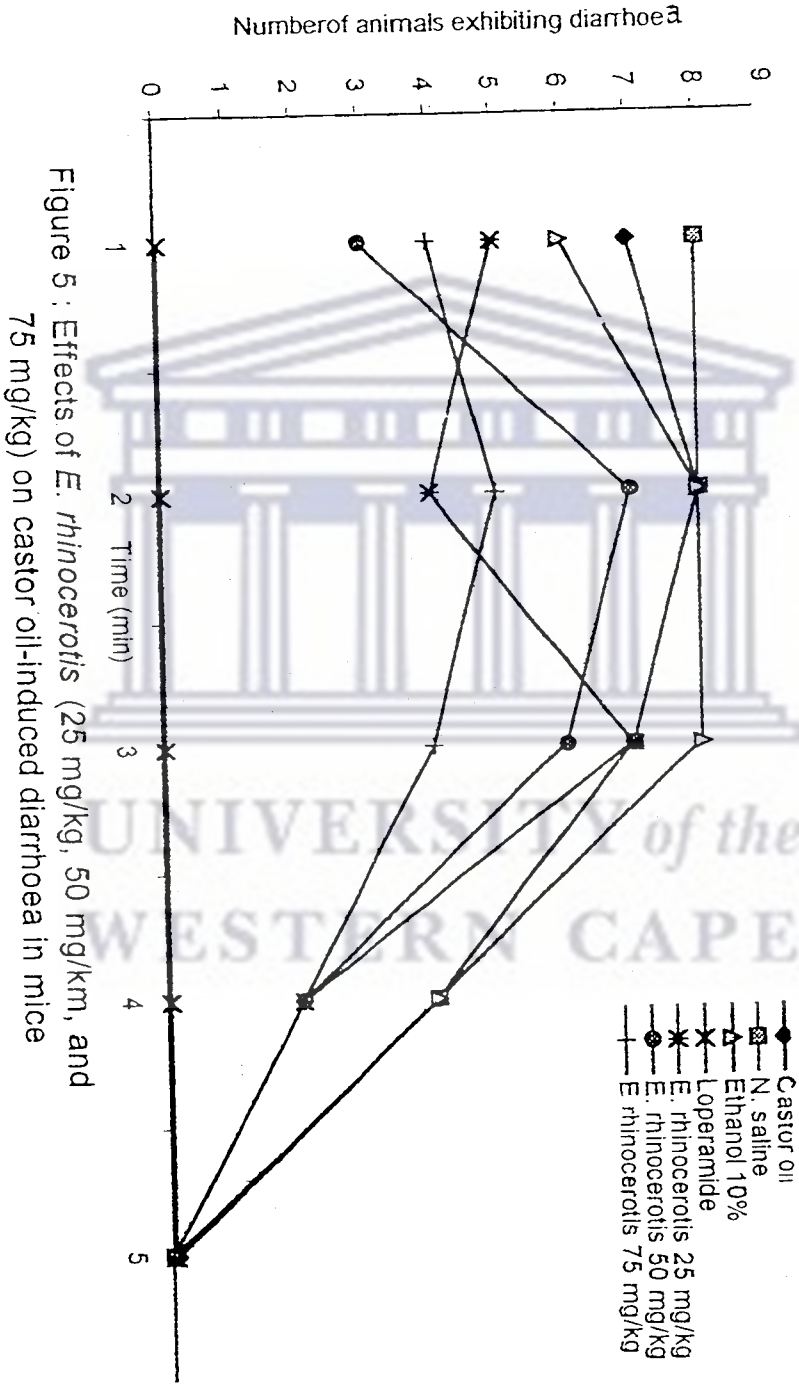


Figure 5 : Effects of *E. rhinocerotis* (25 mg/kg, 50 mg/km, and 75 mg/kg) on castor oil-induced diarrhoea in mice

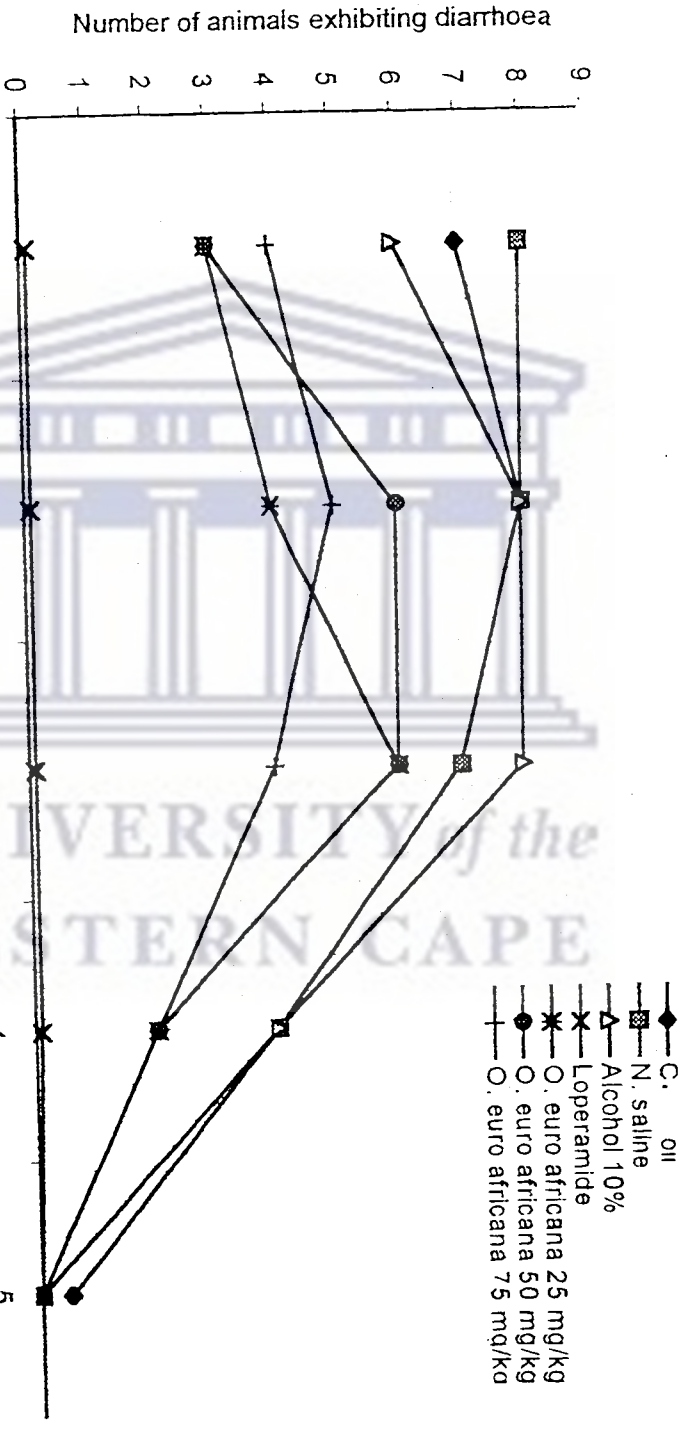


Figure 6 : Effects of O. *euro africana* (25 mg/kg, 50 mg/kg, and 75 mg/kg) on castor oil-induced diarrhoea in mice.

The high performance liquid chromatographic spectrums (Figure7- 12) of the aqueous extracts of *P. triste* shows major peaks (higher height and/or big area) at the following retention times (min): 2.75, 10.15, 11.67, 12.30, 12.61, 15.16, 15.56. These peaks represent the major constituents the extract. A higher and/or a bigger peak area represent the most dominant compound in quantity. The peaks for the most dominant constituents of the extract appeared on 2.75, between 8 and 10.15, at 12.30, and at 15.16. The peak for the standard reference (Rutin) appears at 19.94. No peak on the HPLC spectrum of *P. triste* aqueous extract was found to be identical to the reference standard, Rutin 's peak.

The major peaks of the spectrum of *E. rhinocerotis* aqueous extract appeared at: 10.27, 10.59, 18.04, 18.27, 21.53, 22.15 and 22.48. They represent the major constituents, in quantity, of the extract. . The peak for the standard reference, Rutin, appeared at 19.60. No peak of the HPLC of *E. rhinocerotis* aqueous extract was found to be identical to the standard reference's, Rutin peak.

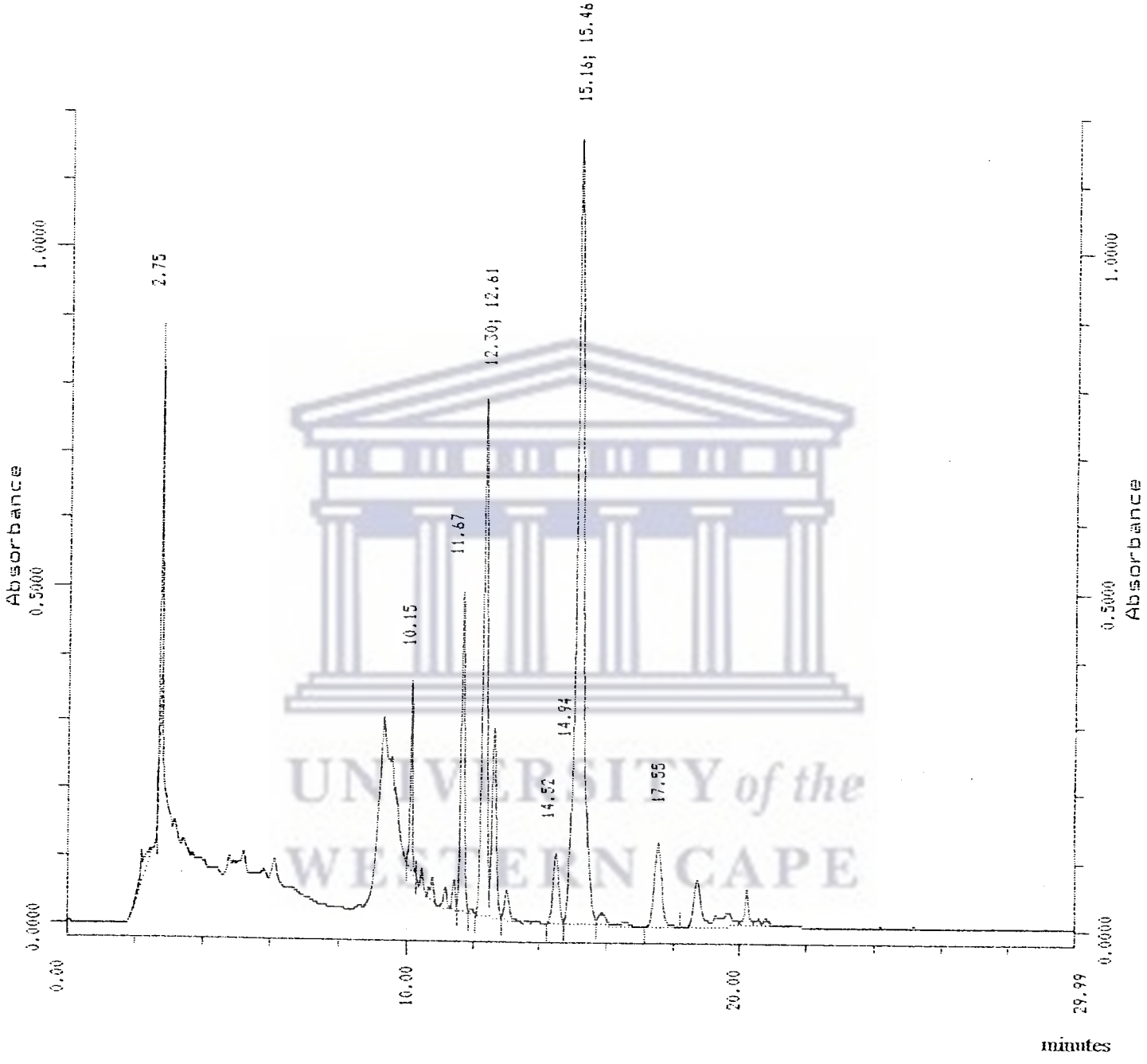


Figure 7 : HPLC spectrum of *Pelargonium triste* Aqueous extract.

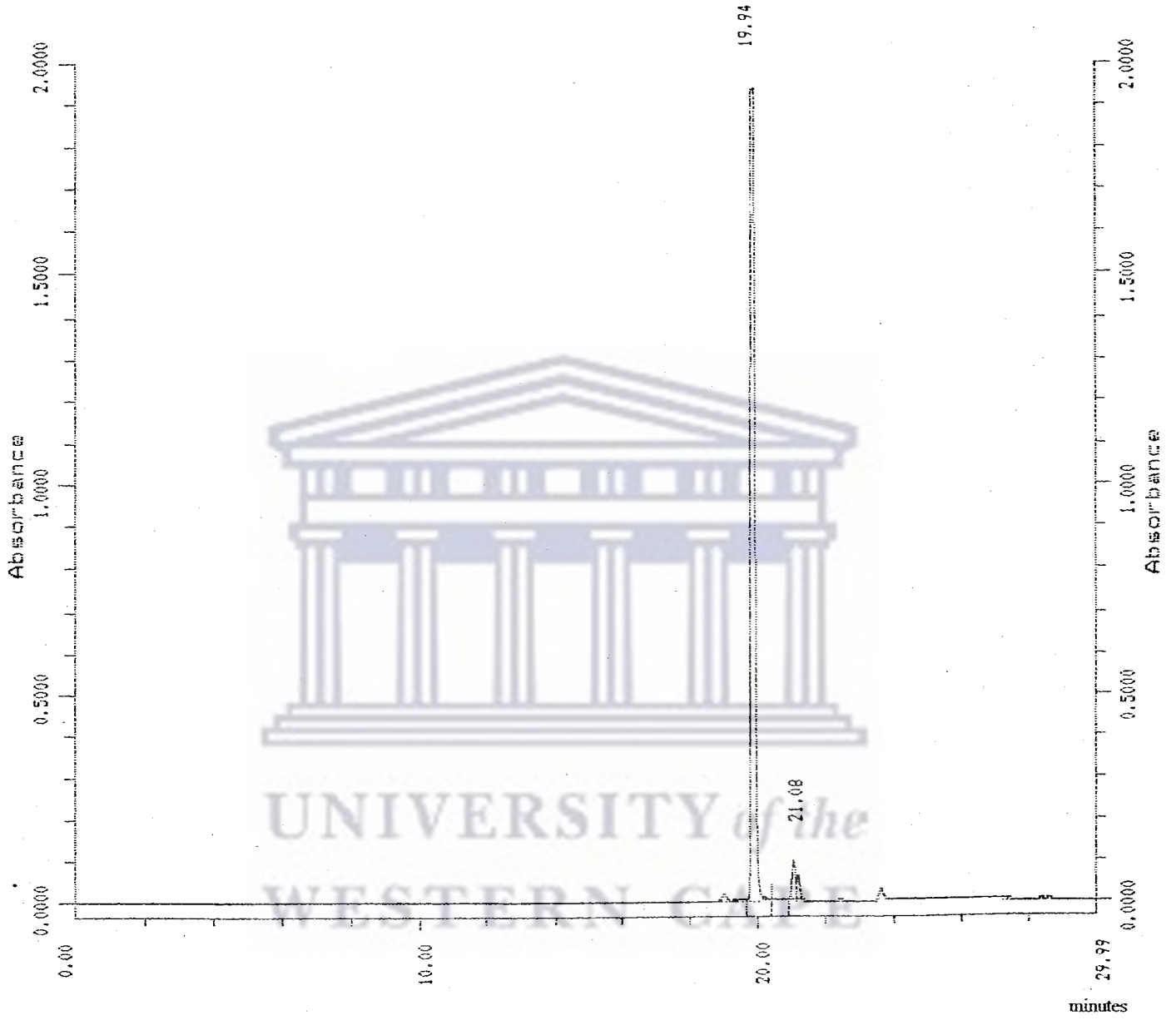


Figure 8 : HPLC spectrum of standard reference, Rutin for *Pelargonim triste* aqueous extract

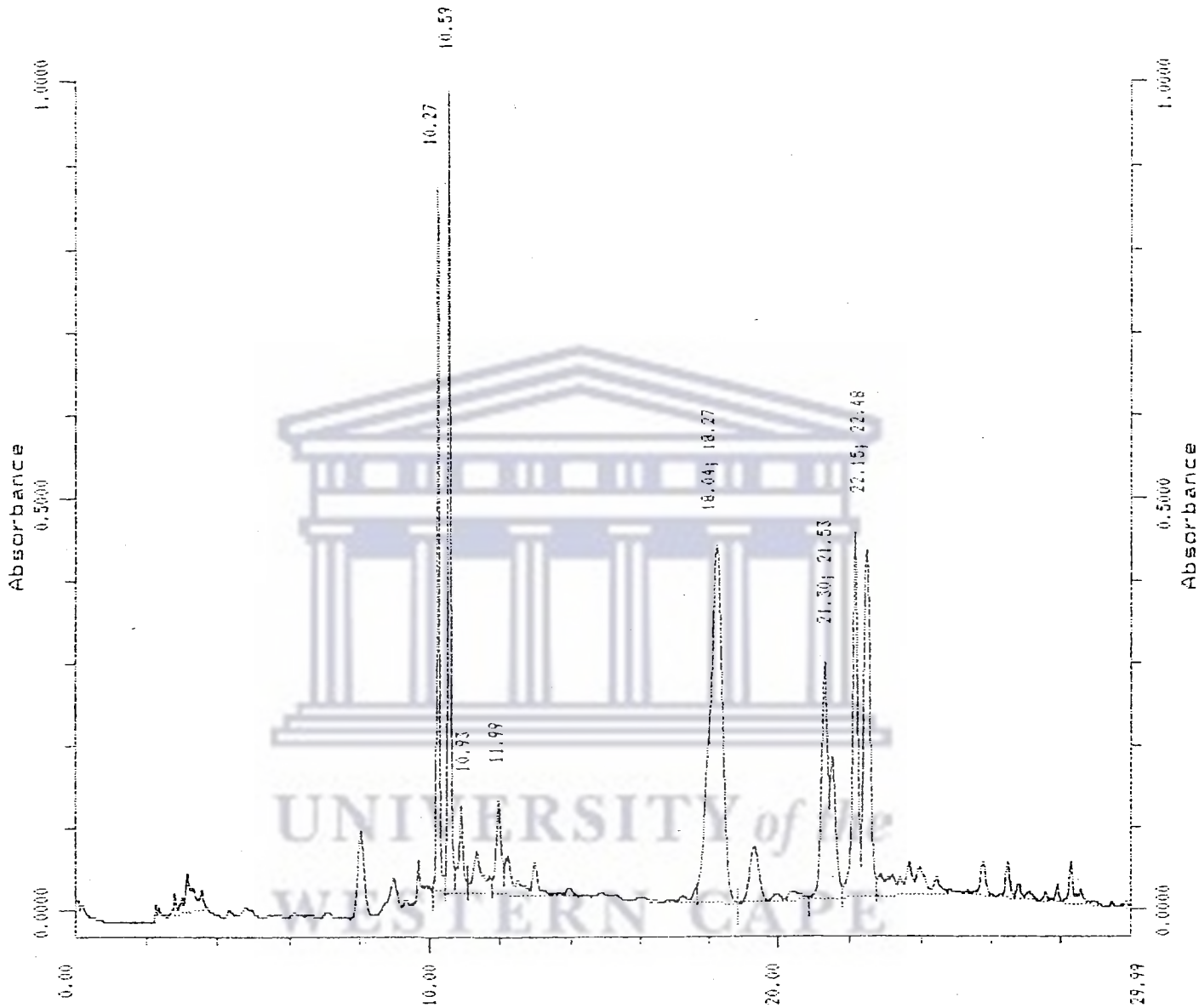


Figure 9 : HPLC spectrum of *Elytropappus rhinocerotis* aqueous extract

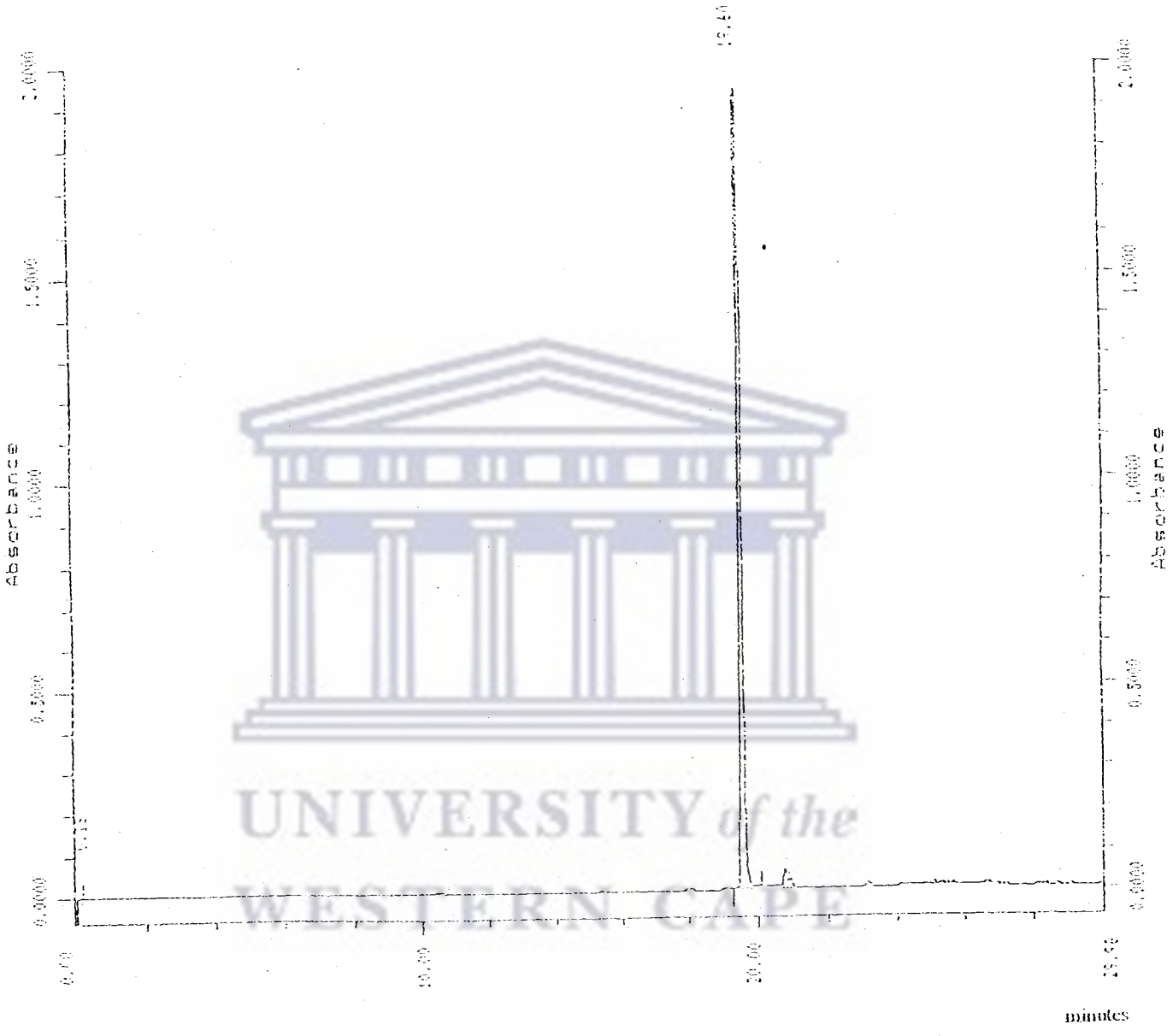


Figure 10 : HPLC sperctrum of standanrd reference, Rutin for *Elytropappus rhinocerotis* aqueous extract

Figure 12 : HPLC spectrum of standard reference, Rutin for *Olea europaea africana* aqueous extract

The phytochemical analysis indicated that crude aqueous extracts of *P. triste* (fleshy tubers) contain saponins, tannins, and reducing sugars.

E. rhinocerotis (leaves + young branches) was found to contain saponins, tannins, and cardiac glycosides, whereas *O. euro africana* was found to contain saponins, tannins, reducing sugars, and anthraquinones.

For *O. euro. africana* aqueous extract the peaks for the major constituents appeared at 22.35, 22.68, 22.84, 24.52 and 24.71, and that of the standard reference, Rutin at 21.47. No peak of the HPLC spectrum of the plant' s extract was found to identical to the Rutin.

Furthermore, the HPLC spectrums obtained show that all the plant extracts do not have similar chemical compositions and their constituents are not structurally identical, though may be of the same phytochemical groups such as tannins, and saponins

4.4 CONCLUSION

In conclusion, data obtained from this study have indicated that the infusions of *P. triste* (fleshy tubers), *E. rhinocerotis* (young branches), and *O. euro. africana* (leaves and steams) possess potential antidiarrhoeal properties, which explain their use by traditional medicine practitioners in Western Cape to combat diarrhoea. Crude aqueous extracts were used for this study in order to simulate the formulations generally used by the traditional medicine practitioners.

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