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

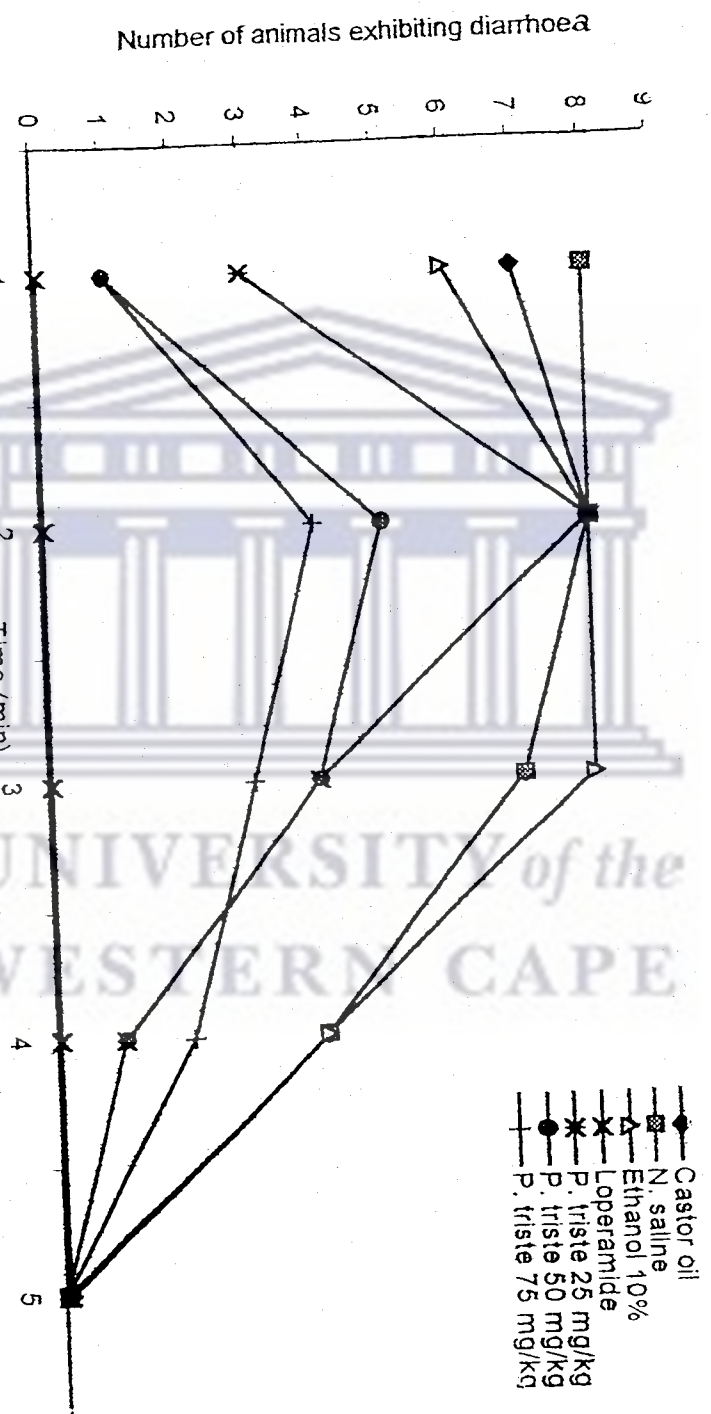

Figure 4 : Effects of *P. triste* (25mg/kg, 50 mg/kg, 75 mg/kg) on castor oil-induced diarrhoea in mice.

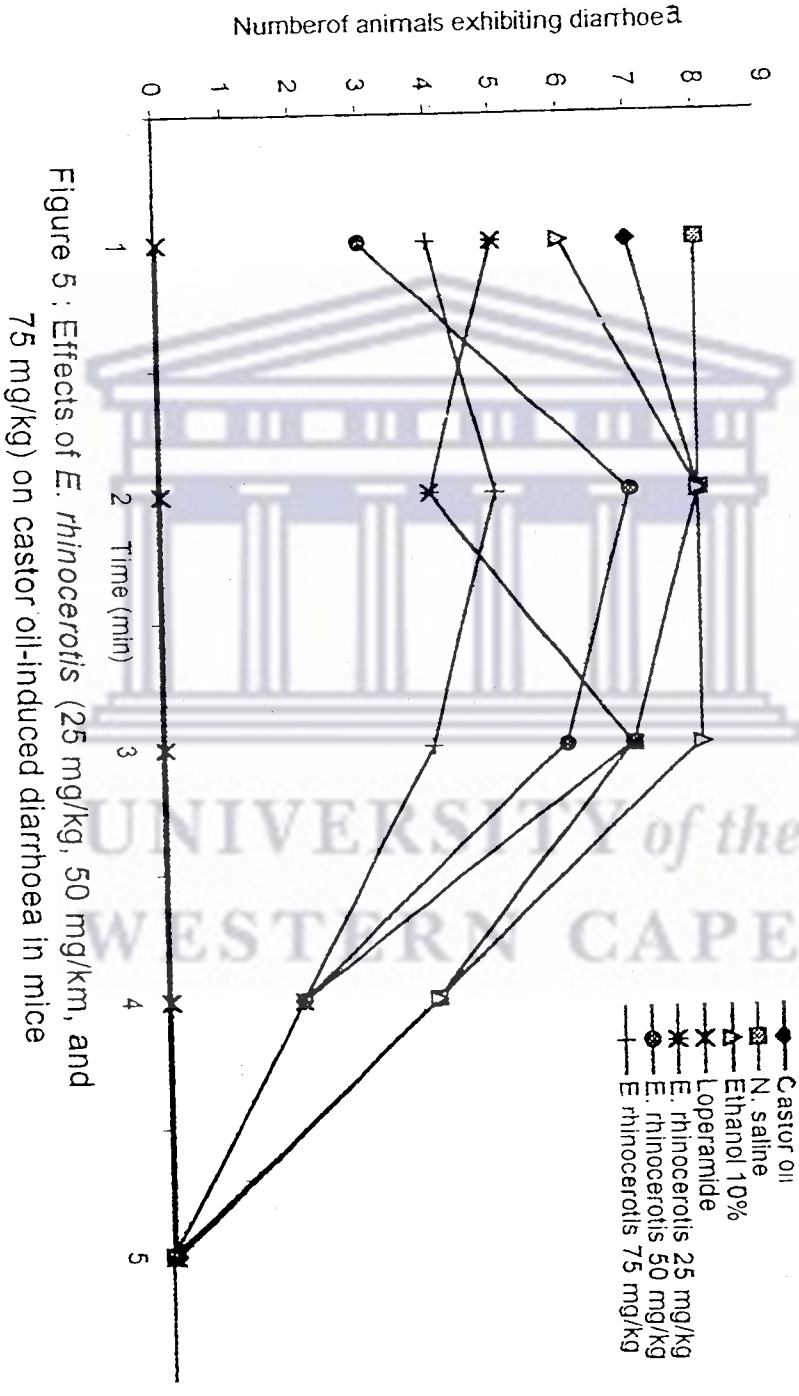

Figure 5 : Effects of *E. rhinocerotis* (25 mg/kg, 50 mg/km, and 75 mg/kg) on castor oil-induced diarrhoea in mice

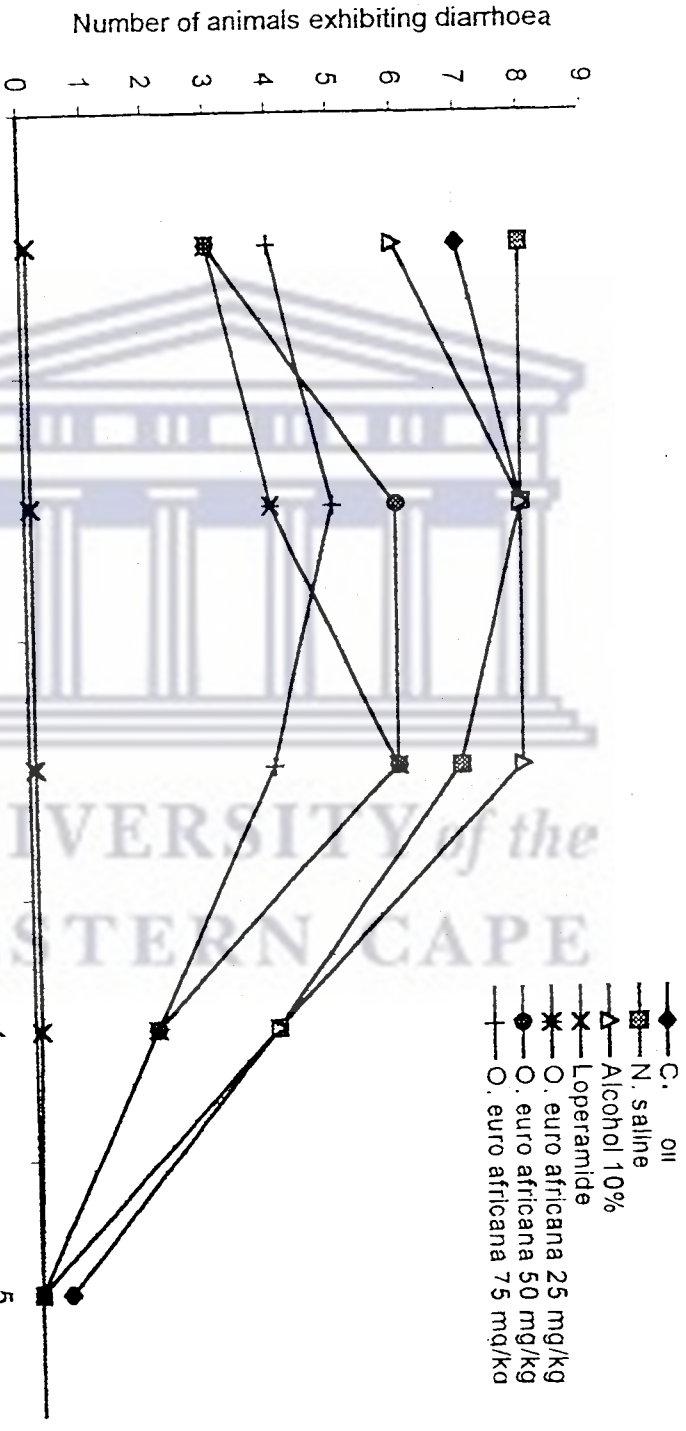

Figure 6 : Effects of O. *euro africana* (25 mg/kg, 50 mg/kg, and 75 mg/kg) on castor oil-induced diarrhoea in mice.

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.

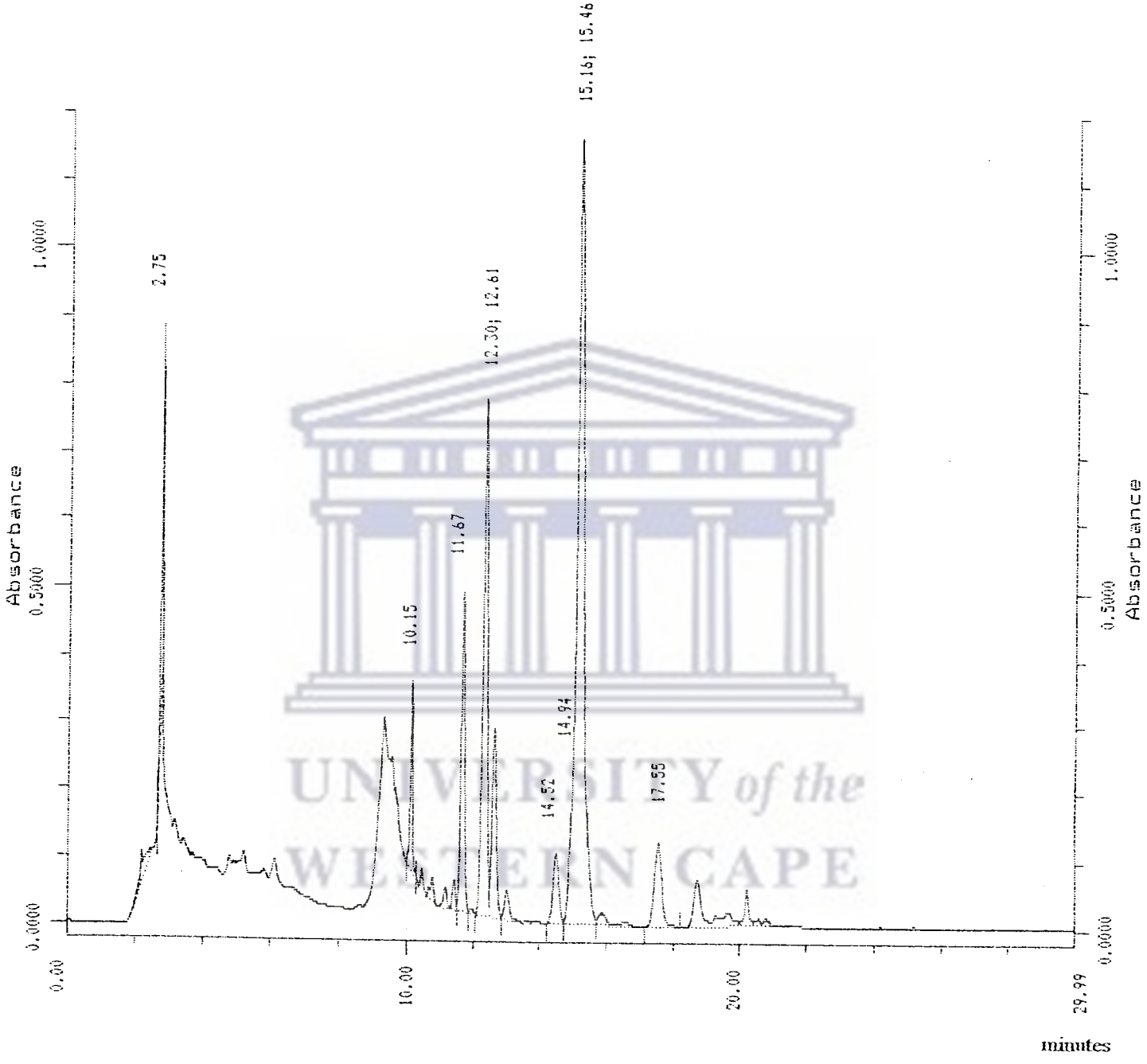

Figure 7 : HPLC spectrum of *Pelargonium triste* Aqueous extract.

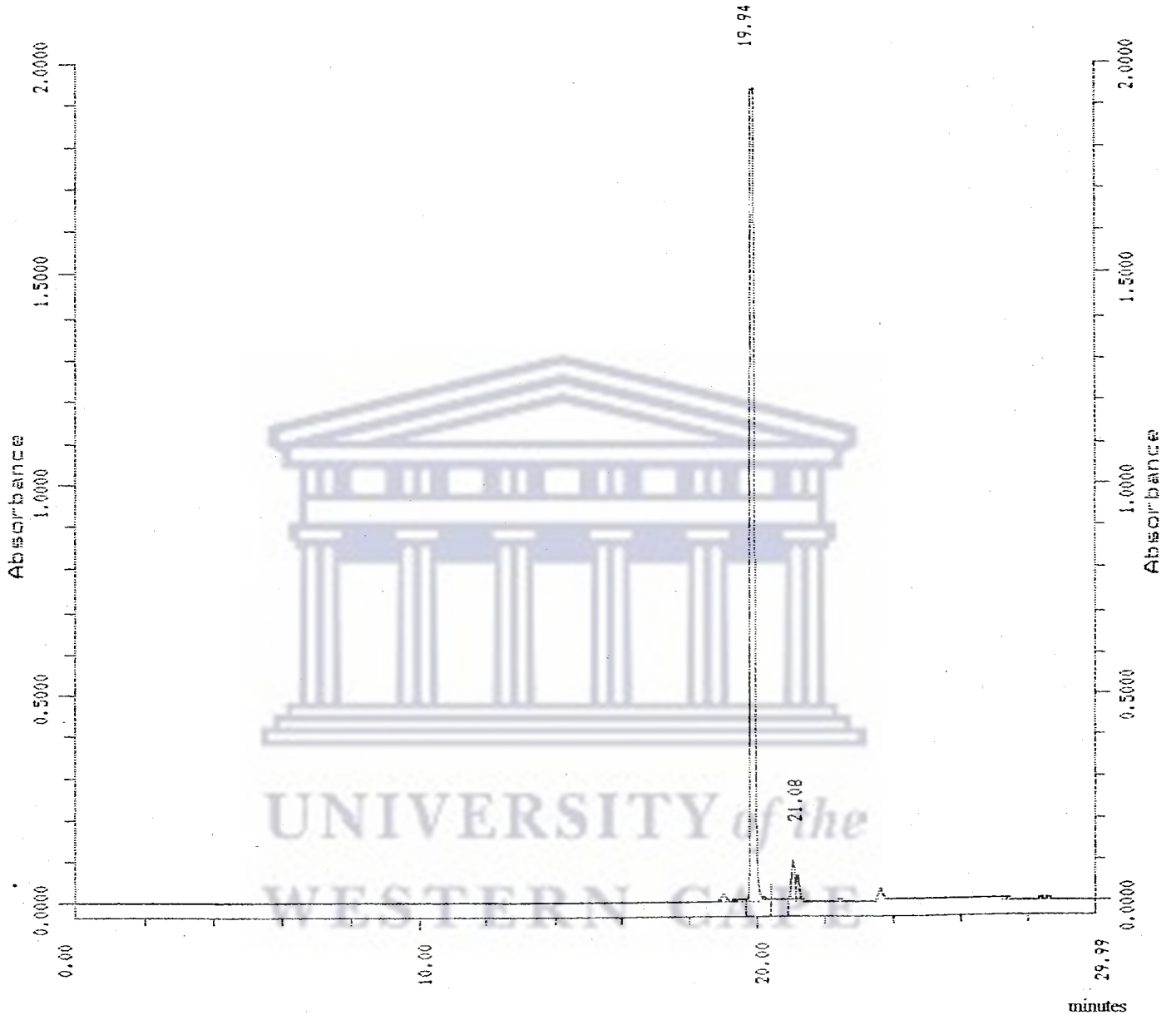

Figure 8 : HPLC spectrum of standard reference, Rutin for *Pelargonim triste* aqueous extract

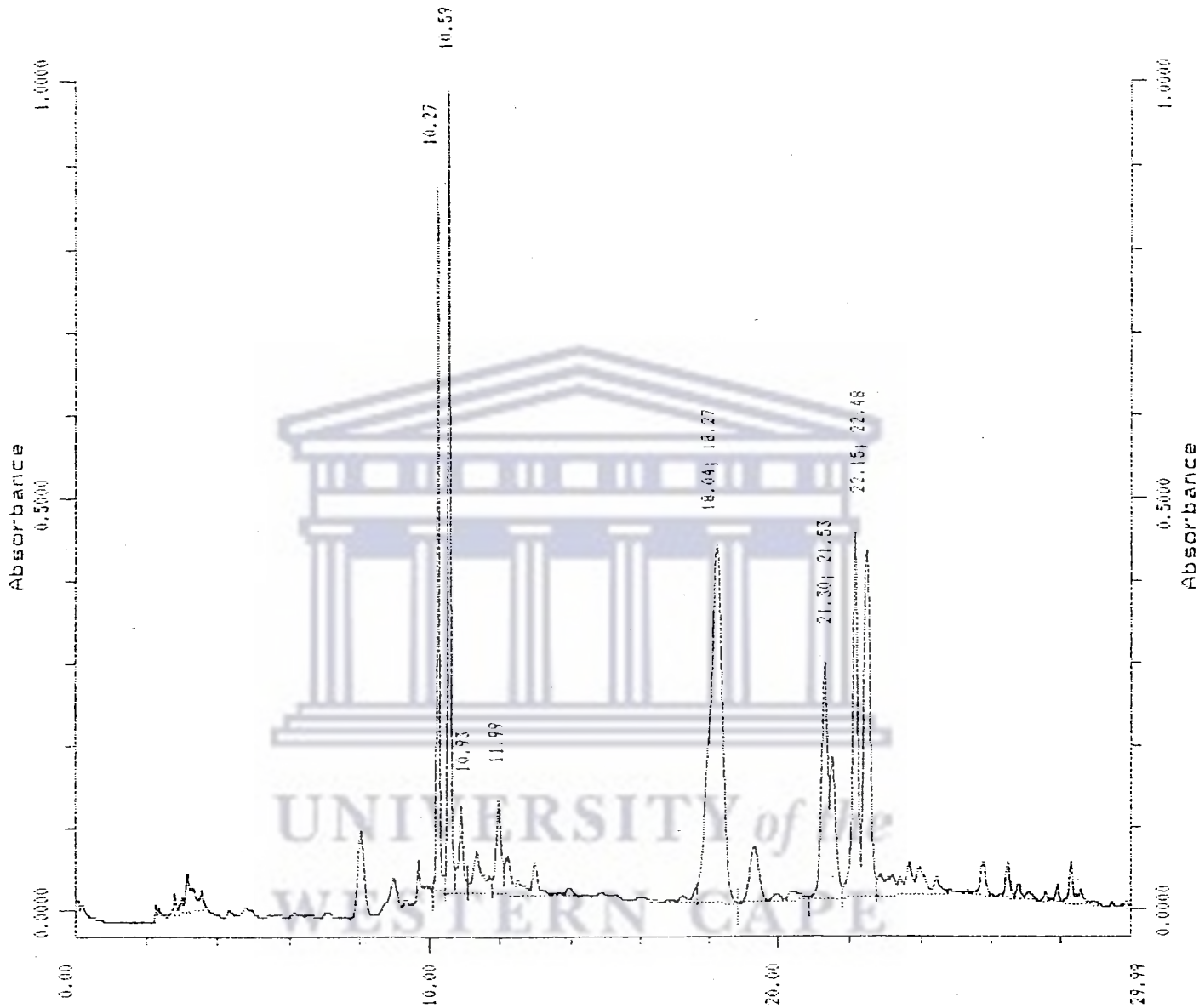

Figure 9 : HPLC spectrum of *Elytropappus rhinocerotis* aqueous extract

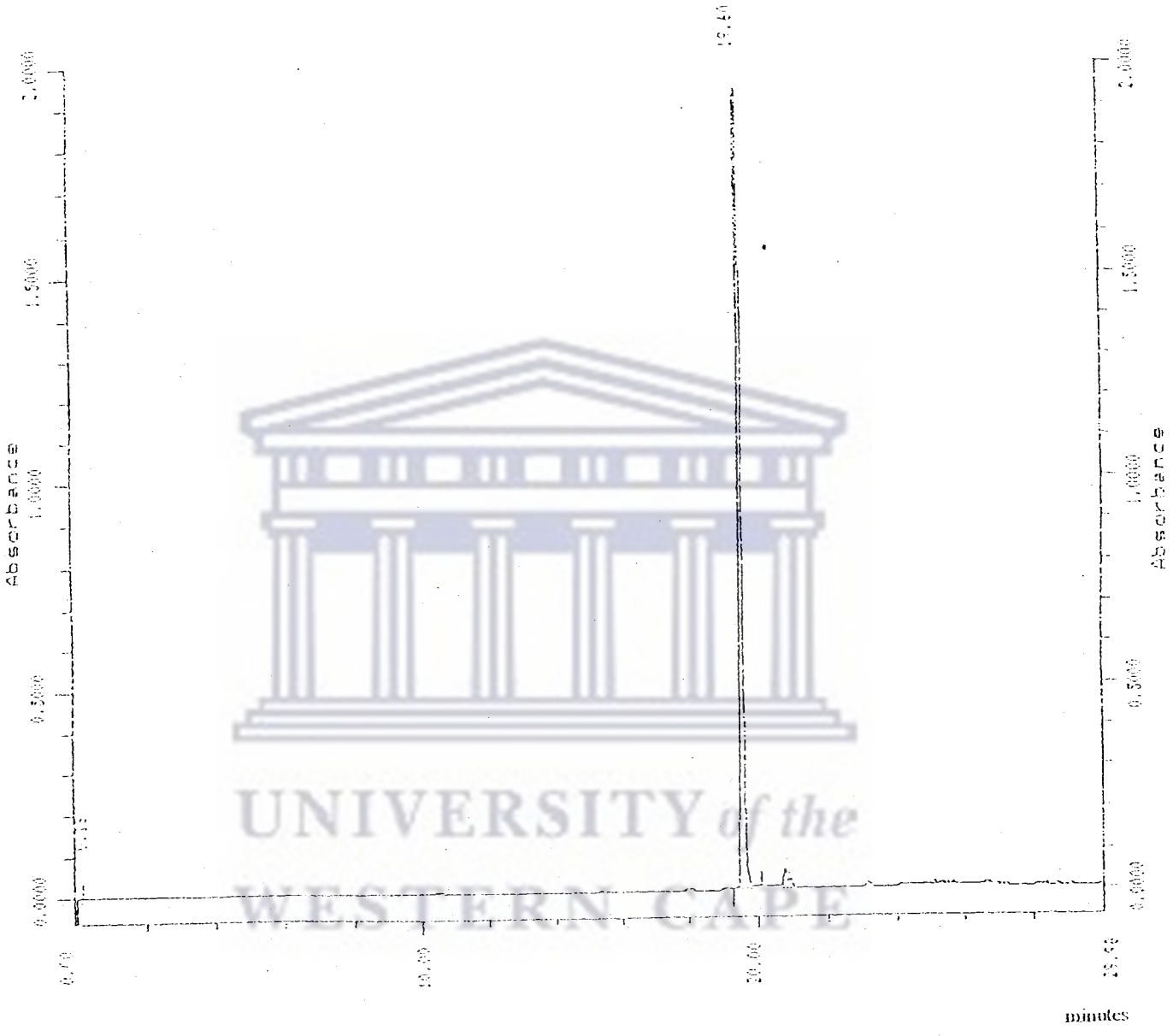

Figure 10 : HPLC sperctrum of standanrd reference, Rutin for *Elytropappus rhinocerotis* aqueous extract

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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