Table 3. Effect of leaf aqueous extract of *Syzygium cordatum* on castor-oil induced intestinal fluid accumulation in mice

Treatment groups (mg/kg)	Intestinal fluid volume (ml) Mean ± SEM		Percentage inhibition (%)
PS			
0.3 ml	1.42	0.19	
Syzygium cordatum			
3.125	0.58**	0.04	59.15
6.25	0.83*	0.15	41.55
12.50	0.80*	0.02	43.66
25.00	0.80*	0.05	43.66
50.00	0.73*	0.05	48.59
Loperamide			
20.00	0.67**	0.07	52.82

*p<0.01, **p<0.001 vs castor oil (1.5 ml, p.o.) control. ANOVA (n=6).

of fasted normal rats throughout the 4 h period of observation. *Syzygium cordatum* (3.125-6.25 mg/kg, p.o.) did not significantly affect the blood glucose concentration of fasted normal rats throughout the 4 h of observation. 12.50-50 mg/kg (p.o.) of *Syzygium cordatum* significantly reduced the blood glucose concentration of fasted normal rats from the second to the fourth hour of observation with a percentage maximal reduction of 28.60-32.79%. Chlorpropamide (250 mg/kg, p.o.) significantly reduced the blood glucose concentration of fasted normal rats from the first hour through the fourth hour of observation and the percentage maximal reduction was 43.26% (Table 4).

Table 4. Effect of leaf aqueous extract of *Syzygium cordatum* on blood glucose concentrations (mmol/l) of normoglycaemic (normal) rats

Treatment groups (mg/kg)	Before treatment	After treatment			Maximal reduction	Percentage maximal reduction (%)
	0 h	1 h	2 h	4 h		
PS						
0.3 ml	6.18 ± 0.24	5.82 ± 0.20	5.65 ± 0.18	6.10 ± 0.20	0.53	8.58
Syzygium cordatum						
3.125	6.67 ± 0.21	5.90 ± 0.13	5.57 ± 0.14	5.72 ± 0.27	1.10	16.49
6.25	6.75 ± 0.25	6.03 ± 0.21	5.37 ± 0.18	5.80 ± 0.24	1.38	20.44
12.50	6.33 ± 0.56	5.98 ± 0.22	4.73 ± 0.32*	4.52 ± 0.38**	1.81	28.60
25.00	6.80 ± 0.20	5.92 ± 0.32	4.75 ± 0.15*	4.60 ± 0.20**	2.20	32.35
50.00	6.80 ± 0.29	5.58 ± 0.29	4.62 ± 0.12*	4.57 ± 0.42**	2.23	32.79
Chlopropamide						
250	6.75 ± 0.40	4.85 ± 0.29*	3.83 ± 0.37***	3.87 ± 0.35***	2.92	43.26

*p<0.05, **p<0.01, ***p<0.001 vs physiological saline (0.3 ml, p.o.) control. ANOVA (n=6).

Values are expressed as mean ± SEM. PS: Physiological saline

4.5 Effect of leaf aqueous extract of *Syzygium cordatum* on blood glucose concentrations (mmol/l) of streptozotocin-treated diabetic rats

Streptozotocin (90 mg/kg, p.o.) raised the blood glucose concentration of fasted animals to 21.40 ± 1.02 mmol/l. 0.3 ml (p.o.) of physiological saline did not significantly affect the blood glucose concentration of fasted diabetic rats throughout the 4 h of observation. 3.125 mg/kg (p.o.) of *Syzygium cordatum* did not significantly alter the blood glucose concentration

of the fasted diabetic rats throughout the 4 h of observation. *Syzygium cordatum* (6.25 mg/kg, p.o.) significantly reduced the blood glucose of the fasted diabetic rats in the 4th h of observation with a percentage maximal reduction of 23.37%. *Syzygium cordatum* (12.50-25 mg/kg, p.o.) significantly reduced the blood glucose concentration of fasted diabetic rats from the 2nd to the 4th h of observation with a percentage maximal reduction of 33.18-35.54%. 50 mg/kg (p.o.) of *S. cordatum* significantly reduced the blood glucose concentration of fasted diabetic rats from the 1st h through to the 4th h of observation with a percentage maximal reduction of 33.37%. Chlorpropamide (250 mg/kg, p.o.) profoundly reduced the blood glucose concentration of fasted diabetic rats from the 1st h through the 4th h of observation with a percentage maximal reduction of 93.42% (Table 5).

4.6 Acute toxicity test

The leaf aqueous extract of *Syzygium cordatum* (200, 400, 800, 1200, 1600, 2000, 2400, 2800, 3200, 3600 and 4000 mg/kg) administered orally to mice did not cause any death to or any acute toxicity symptoms in the animals in all the doses used. The highest dose tested being 4000 mg/kg should be the no-adverse-effect-level (NOAEL). The LD_{50} value for the plant species should, therefore, be greater than 4000 mg/kg (p.o.).

4.7 Phytochemical analysis

The phytochemical screening methods used to determine the active constituents present in the leaves of *Syzygium cordatum* tested positive for alkaloids, tannins, flavonoids, reducing sugars, triterpene steroids and saponins. However, no quinones were present (Table 6).

Table 5. Effect of leaf aqueous extract of *Syzygium cordatum* on blood glucose concentrations (mmol/l) of streptozotocin-treated diabetic rats

Treatment groups (mg/kg)	Before treatment 0 h	After treatment 1 h	2 h	4 h	Maximal reduction	Percentage maximal reduction (%)
PS						
0.3 ml	21.40 ± 1.02	23.67 ± 1.26	23.67 ± 0.89	23.88 ± 1.00		
Syzygium Cordatum						
3.125	23.37 ± 1.10	23.78 ± 0.66	23.20 ± 0.60	22.95 ± 0.47	0.83	3.49
6.25	22.68 ± 1.04	22.43 ± 0.52	20.77 ± 0.55	17.38 ± 1.11*	5.30	23.37
12.50	20.32 ± 0.59	21.28 ± 1.14	15.25 ± 0.78*	14.22 ± 1.80*	7.06	33.18
25.00	20.17 ± 0.57	21.41 ± 1.53	14.12 ± 0.12*	13.80 ± 0.93*	7.61	35.54
50.00	20.53 ± 2.08	14.42 ± 0.87*	14.07 ± 0.68*	13.68 ± 0.89*	6.85	33.37
Chlopropamide						
250	23.23 ± 0.94	15.55 ± 1.34*	6.38 ± 1.12*	4.05 ± 0.24*	19.18	93.42

*p<0.001 vs streptozotocin (90 mg/kg, i.p.) control. ANOVA (n=6).

Values are expressed as mean ± SEM.

PS: Physiological saline

Table 6. Phytochemical screening of the leaf aqueous extract of *Syzygium cordatum*

Compound	Result
Tannins	+
Saponins	+
Alkaloids	+
Flavonoids	+
Triterpene steroids	+
Quinones	-
Reducing sugars	+

+ (positive): Present, - (negative): Absent

CHAPTER 5

DISCUSSION

Diarrhoea and diabetes are debilitating conditions known to afflict so many people worldwide and especially on the African continent (Weber,1976; Syder and Merson, 1982; Rother, 2007). Modern or orthodox medicines have provided adequate management and treatment of the conditions. It is a well established fact that about 80% of the population especially in developing countries rely on herbal medicines for their healthcare need. The reasons range from low cost to availability of these medicines and their use depends on ancestral experience. WHO has also urged various governments especially those of the developing countries, to include in their healthcare programmes those herbal medicines with proven safety and efficacy (Marin-Bettolo, 1980; Amos et al., 2001). *Syzygium cordatum* is one medicinal plant used by traditional medicine practitioners in South Africa to treat various ailments including diarrhoea and diabetes (Van Wyk et al., 1997). In order to scientifically scrutinized the claims by traditional medicine practitioners of the therapeutic success of the plant species, this project studied the antidiarrhoeal and antidiabetic activities of *Syzygium cordatum* in mice and rats respectively.

In this study, the plant extract, up to the highest dose (4000 mg/kg, p.o.) used in the acute toxicity test, did not cause any death to or acute toxicity symptoms in the mice. The LD_{50}, therefore, may be greater than 4000 mg/kg (p.o.). This relatively high LD_{50} shows that the plant extract is non-toxic and/or safe in mice. Traditional medicine practitioners are known to use the plant for treatment in the form of infusion (Van Wyk et al., 1997). However, this study did not ascertain the doses used by the practitioners for such treatments.

The pharmacological screening results obtained in the present study, show that *S. cordatum*

(3.125-50 mg/kg, p.o.)antagonized the diarrhoea produced by castor oil (0.7 ml, p.o.). Loperamide (20 mg/kg, p.o.) also antagonized the castor oil-induced diarrhoea in mice. Castor oil, an irritant laxative, is thought to produce diarrhoea by being hydrolysed in the upper small intestine to ricinoleic acid which exerts its effects by irritating the mucosa of the gastrointestinal tract, resulting in an increase in intestinal motility (Altman, 2001). Furthermore, ricinoleic acid has been shown to diminish the permeability of sodium and chloride ions and also stimulate the release of prostaglandin, known to cause diarrhoea (Gaginella and Phillips, 1975; Zavala et al., 1998).In addition, the works of Capasso et al. (1994) and Mascolo et al. (1994), on the effect of N^G-nitro-L-arginine methyl esther, an inhibitor of nitric oxide (NO) synthase, on the dissociation of castor oil-induced diarrhoea and mucosal injury in rat, showed that nitric oxide may mediate castor oil-induced diarrhoea.

Loperamide, an opioid derivative and a standard antidiarrhoeal drug, is thought to decrease intestinal motility by binding to mu receptors on neurons in the submucosal neural plexus of the intestinal wall. This leads to the segmental contractions in the colon increasing, the propulsive movement of the small intestine and colon being inhibited and the transit time of the intestinal content being prolonged (Altman, 2001). Loperamide is also known to have an antimuscarinic activity contributing to the inhibition of peristalsis by inhibiting contractions in both the longitudinal and circular muscles (Altman, 2001; Camillen et al., 2002; Waller et al., 2005). In this study, therefore, loperamide may be antagonizing castor oil-induced diarrhoea by decreasing the intestinal motility. Similarly, *Syzygium cordatum* may be said to exert its antidiarrhoeal activity by slowing intestinal motility.

In my study, the leaf aqueous extract of *S. cordatum* significantly antagonized the gastrointestinal transit of charcoal meal and also significantly reduced the castor oil-induced intraluminal accumulation of fluid volume. Similarly, loperamide significantly antagonized

the gastrointestinal transit of charcoal meal and also significantly reduced the castor oil-induced intraluminal accumulation of fluid volume. According to DiCarlo et al. (1994), agents that reduce intestinal motility and secretion may possess antidiarrhoeal activity. Furthermore, Nwafor et al. (2000) have shown that agents that suppress intestinal fluid accumulation may inhibit gastrointestinal functions. The above reports lend support to the suggestion that *S. cordatum* may be exerting its antidiarrhoeal activity by slowing intestinal motility.

The phytochemical analysis of the powdered leaf of *S. cordatum* carried out in this study showed that the plant species contains the following chemical components, tannins, saponins, alkaloids, triterpene steroids, flavonoids and reducing sugars. Several studies have shown tannins to have antidiarrhoeal activity. Tannin containing drugs have been used for the treatment of diarrhoea and other related disorders (Frei et al., 1998; Bruneton, 1999). Astringents such as tannins have been known since the last century to have antisecretory effect in the gastrointestinal tract and have been used to treat diarrhoea (Farthing). It is probable therefore, that the presence of tannins in the plant species as shown by the phytochemical analysis, may contribute to the antidiarrhoeal activity of *S. cordatum*.

Syzygium cordatum was shown in the present study to have an antidiabetic activity. Chlorpropamide, a sulphonylurea, and an oral antidiabetic agent used for the treatment of Type 2 or non-insulin dependent diabetes (NIDD), was used as a standard drug in this study. Chlorpropamide is thought to act by stimulating and increasing the release of endogenous insulin from the pancreatic beta cells of the Islet of Langerhans (Waller et al., 2005a; Rang et al., 2008). Diabetes, in this study, was induced using streptozotocin (STZ). Streptozotocin is thought to produce diabetes by a rapid depletion of pancreatic beta cells and thereby, reducing insulin release and causing hyperglycaemia (Mahomed and Ojewole, 2003). Thus,

the STZ-induced rat diabetes model has the hallmark of non-insulin dependent diabetes or Type 2 diabetes. In this study, *S. cordatum* (12.5-50 mg/kg, p.o.) and chlorpropamide (250 mg/kg, p.o.) significantly reduced the blood glucose concentration of fasted normal rats while 0.3 ml (p.o.) of physiological saline did not alter the blood glucose concentration of the fasted normal rats. Similarly, both the leaf aqueous extract of *S. cordatum* (12.5-50 mg/kg, p.o.) and chlorpropamide (250 mg/kg, p.o.) significantly reduced the glucose concentration of diabetic rats treated with streptozotocin. Since chlorpropamide used to treat diabetes, acts by stimulating insulin secretion from pancreatic bête cells and also promoting peripheral glucose uptake and utilization (Waller et al., 2005a; Rang et al., 2008), it is probable that *S. cordatum*, may be acting in a similar manner. The result obtained in this study is in agreement with the study of Musabayane et al. (2005) who showed that *S. cordatum* leaf extract significantly lowered the plasma glucose and hepatic glycogen levels in STZ-induced diabetic rats.

In this study, the phytochemical analysis carried out revealed the presence of alkaloids and flavonoids amongst other chemical metabolites in *S. cordatum*. Punitha et al. (2005) in their study with berberine, an alkaloid, and antidiabetic activity, showed that alkaloids have antidiabetic activity. Dineshkumar et al. (2010) in their study on the antidiabetic and hypolipidemic effects of mahanimbin from *Murraya koenigii* leaves also showed that alkaloids have antidiabetic activity. Flavonoids have also been shown by the study of Ghada et al. (2008) which investigated the antidiabetic and antioxidant activities of major flavonoids of*Cynanchum acutum* L., to have antidiabetic activity. Furthermore, Hule et al. (2011) in their study on the evaluation of the antidiabetic effects of *Elaeocarpus ganitrus* in experimental animals, showed that alkaloids and flavonoids have antidiabetic activities. It is probable therefore, that the flavonoids and alkaloids found in *S. cordatum* may be contributing to its antidiabetic activity.

CHAPTER 6

CONCLUSION

The data obtained in this study indicate that *Syzygium cordatum* have both antidiarrhoeal and antidiabetic activities. This project was not set out to investigate the mechanisms of the antidiarrhoeal and antidiabetic activities of *S. cordatum*. However, various studies as shown above, have shown that castor oil-induced diarrhoea may also involve increase in electrolyte permeability, increased release of prostaglandins and nitric acid mechanism. It is probable therefore, that the antidiarrhoeal activity of *S. cordatum* may involve the inhibition of electrolyte permeability, inhibition of prostaglandin release and inhibition of nitric acid mechanism. The antidiabetic activity may be due to the plant species stimulating the release of insulin from the pancreatic beta cells since streptozotocin used to induce diabetes is known to act by rapidly depleting pancreatic beta cells and thus, reducing insulin release. The role of tannins in the antidiarrhoeal activity and alkaloids and flavonoids in the antidiabetic activity of *S. cordatum* also needs mentioning. The relatively high LD50 of the plant species shows that it is non-toxic and /or safe in mice. These data may justify the use of the plant species by traditional medicine practitioners in the treatment of diarrhoea and diabetes especially Type 2 or non-insulin dependent diabetes. However, further studies on the acute toxicity and the mechanisms of the antidiarrhoeal and antidiabetic activities of *S. cordatum* need to be carried out to enhance the safety and efficacy of the plant species.

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