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Original Research Article

Pharmacodynamic and cytotoxicity effects of Syzygium cordatum {S Ncik, 48 (UZ)} fruit-pulp extract in gastrointestinal tract infections

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Abstract

Purpose: *To evaluate the pharmacodynamic effect and cytotoxicity of S. cordatum pulp extract in the treatment of gastrointestinal tract infections.*
Methods: *The air-dried fruit pulps were ground, extracted with 100 % methanol and screened for phytochemicals. Serial microdilution method was used to determine the antibacterial activity of the extract against Bacillus cereus (ATCC 10102), Staphylococcus aureus (ATCC 25925), Enterococcus hirae (ATCC 8043), Escherichia coli (ATCC 25922), Salmonella typhimurium (ATCC 700030), Klebsiella pneumonia (ATCC 4352), Pseudomonas aeruginosa (ATCC 7700), Vibrio fluvialis (AL 019) and Vibrio vulnificus (AL 042). The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay was used to assess the cytotoxicity of the extract. Antidiarrheal and antimotility activities were evaluated using castor oil-induced diarrhoea model in rats.*
Results: *The extract revealed the presence of alkaloids, cardiac glycosides, flavonoids, saponins and terpenoids. Total phenolic content was 16.4 ± 1.8 µg/mg. The extract exhibited antibacterial activity with minimum inhibitory concentration (MIC) of as low as 3.13 mg/ml against B. cereus (ATCC 10102), S. aureus (ATCC 25925), E. hirae (ATCC 8043), P. aeruginosa (ATCC 7700) and K. pneumonia (ATCC 4352). Median inhibitory concentration (IC_{50}) of 92 µg/ml and therapeutic index of 0.1 - 0.3 were exerted by the extract. In vivo antidiarrheal activity was 49 % at extract dose of 400 mg/kg, which was much higher than that of the control (0 %).*
Conclusion: *The fruit-pulp extract of Syzyhium cordatum has both antibacterial, antimotility and antidiarrheal activities, and may therefore be clinically safe for use at low concentrations as an antidiarrheal agent.*

Keywords: *Syzyhium cordatum, Antibacterial, Antidiarrheal, Antimotility, Cytotoxicity*

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INTRODUCTION

Gastrointestinal tract (GIT) infections are the major cause of high morbidity and mortality rates, especially in the developing countries. More than 1.5 billion episodes of GIT infections that result in more than 3 million deaths are reported annually in the developing countries [1]. Twenty percent of deaths of children under the age of five years are attributable to GIT infections per year. In the Republic of South Africa (RSA), the South African National Aids Council (SANAC), reported

50 471 children's deaths under the age of five and GIT infections were the second leading cause of these deaths [2]. The microbial resistance to current antibiotics, the high cost and side effects of allopathic medicine contribute to the high prevalence of GIT infections.

Epidemiological studies have approved consumption of fruits in preferred amounts (400-500 grams per day) to be adequate to save approximately 2.7 million people from chronic infections including GIT infections worldwide per year [3]. Fruits are not only excellent sources of nutrients but therapeutic phytochemicals as well. Phytochemicals have been revealed to boost the immune system, modulate detoxifying enzymes, facilitate better nutrient absorption and for their strong antimicrobial, antidiarrhoeal and gastroprotective properties in humans [4]. However, fruits are rarely used as medicine.

Fruit extracts are generally accepted as non-toxic. Any plant material used as food or medicine has to be proven to be nontoxic [5]. This is brought by the fact that the extracts may show a therapeutic effect at one point and toxic effect at another. Toxic fruit extracts can cause damage to immune cells' function, cell lysis and even cell-death. This can directly or indirectly cause more severe occurrences of GIT infections. *Syzigium cordatum* is reported to have significantly high quantity of various phytochemicals. *S. cordatum* extracts (with the exception of the fruit extracts) have been utilised in the treatment of mild diabetes mellitus, haemorrhage, dysentery and gastrointestinal disorders [6]. In this study, *S. cordatum* fruit pulp extract was evaluated for its phytochemical content and antibacterial activity. The cytotoxicity and pharmacodynamics of the extract was also assessed against GIT infections causing bacteria.

EXPERIMENTAL

Chemicals

All the chemicals used including the solvents, were of analytical grade from Sigma-Aldrich Co., Ltd (Steinheim, Germany).

Plant materials

Fresh fruits of *S. cordatum* were collected from KwaDlangezwa area in the city of Umhlathuze, KwaZulu-Natal Province, South Africa (28 °45 ′S 31 °54 ′E). The fruits were authenticated by Prof De wet H, Department of Botany, University of Zululand, KwaDlangezwa. A voucher specimen was deposited at the University Herbarium {*S.Ncik*, 48 (UZ)}. The fruits were thoroughly washed and manually separated from their seeds. The fruit pulps were dried at room temperature, ground to powder form and stored in sterile brown glass bottles at 4 °C until used.

Preparation of the extract

The air-dried and pulverized fruit pulps were extracted with methanol (1 : 4 w/v). The resultant extract was concentrated to dryness under reduced pressure in a rotary evaporator (45 °C) to yield dried methanol extract, which was (10 %) of the starting material. Dried extract was re-dissolved in methanol for further experiments.

Phytochemical screening

Well established methods were used to screen the crude extract [7].

Determination of total phenolic content

The total phenolic content was determined according to the existing method [8]. An aliquot (0.2 ml) of 500 µg/ml methanolic fruit pulp extract was made up to 1.0 ml with distilled water. 0.5 ml of Folin-Ciocalteau reagent (1 N) was added, followed by 2.5 ml of sodium carbonate solution (20 %). The mixture was shaken properly and incubated at room temperature for 40 min. The absorbance of the blue-coloured complex formed was spectrophotometrically measured at 725 nm against the appropriate blank. The total phenolic content was determined from the standard curve of tannic acid and expressed as equivalents of tannic acid (µg/ml).

Evaluation of antimicrobial activity

Bacterial strains known to cause GIT infections used in this study included: *Bacillus cereus* (ATCC 10102*), Staphylococcus aureus* (ATCC 25925*), Enterococcus hirae* (ATCC 8043), *Escherichia coli* (ATCC 25922), *Salmonella typhimurium* (ATCC 700030), *Pseudomonas aeruginosa* (ATCC 7700), *Klebsiella pneumonia* (ATCC 4352), *Vibrio fluvialis* (AL 019) and *Vibrio vulnificus* (AL 042).

Resuscitation of bacterial strains

The selected bacteria were inoculated into sterile nutrient broth and incubated overnight at 37 °C. Thereafter, 1 ml from each of the bacterial cultures was pipetted into 9 ml of fresh prepared nutrient broth in separate test tubes. The test tubes were then incubated at 37 °C overnight. After an overnight incubation, absorbance of the bacterial strains was read in the

spectrophotometer (600 nm) for determination of their turbidity. The turbidity of the resulting suspensions was diluted with nutrient broth to obtain an absorbance of 0.132. This absorbance was taken as comparable to 0.5 McFarland turbidity standard. The turbidity was estimated to be equivalent to 1.5 x 10^6 colony forming unit (CFU)/ml [9].

Determination of minimum inhibitory concentration (MIC)

A serial microdilution method was adopted as described [10], to evaluate the minimal inhibitory concentration (MIC) of the fruit pulp extract. Ninety-six well microplate was used to quantitatively determine the MIC of the extract. The sterile nutrient broth (50 µl) was added to all the wells of the 96-well microplates and 50 µl of the extract (50 mg/ml, in 10 % DMSO) was poured in the wells in the first rows and mixed well. The mixture (50 µl) was removed from all the wells in row A to perform a 3-fold serial dilution down the columns. The last 50 µl, in the last column was discarded so that the total volume solution of each well was 50 µl. The selected bacterial strains (50 µl) were transferred into the corresponding wells. Ten percent of DMSO was used as a negative control while ciprofloxacin (20 µg/ml) was used as a positive control. The plate was covered and incubated at 37 °C overnight. A 0.2 mg/ml of p-iodonitrotetrazodium violet (INT) solution was utilised after the incubation period. A 40 µl of 0.2 mg/ml INT solution was added to each well and incubated at 37 °C for 30 min. A reddish colour was the result of INT being reduced by the metabolic activity of bacteria to formazan. The clear colour was the indication of the absence of bacterial activity since the INT was not broken-down to form formazan. The tests were replicated three times and the mean values reported. The MIC was taken as the lowest concentration of the extract required to inhibit the bacterial growth.

Evaluation of minimum bactericidal concentration (MBC)

Agar dilution method was used to determine MBC of the extract [10]. MBC was evaluated by removing a loopful of each culture from the wells that had no bacterial growth. They were streaked on different sterile nutrient agar plates. The agar plates were incubated at 37 °C for 12 h. The lowest concentration of the fruit pulp extract that exhibited the complete killing of the selected bacterial test species was considered as the MBC.

Cell cytotoxicity assay

Human colorectal adenocarcinoma cells (Caco-2) were cultured on minimum essential medium (MEM) supplemented with 10 % foetal bovine serum (FBS), glutamine (2 mM) and 1 % penicillin-streptomycin in static flask. The cells were incubated in a humidified atmosphere at 37 °C in 5 % CO_2 for 24 h. The cells were plated in a 96-well-plate with cell suspensions of 1 × 10^5 cells/ml concentrations. The cells were allowed to attach for 48 h before being seeded with different concentrations from the crude extracts (150 mg/ml) administered in media containing 1 % of FBS and incubated for 48 h. After 48 h incubation, the cell viability was determined by removing the old medium and adding the (Merck) tetrazolium salt as a cytotoxicity indicator.

Subsequently, 100 µl of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) (MTT) (5 mg/ml) in phosphate buffered saline (PBS) was added to each well and incubated at 37 °C for 4 h. The insoluble purple formazan from yellowish MTT reagent by enzymatic reduction was dissolved in DMSO after abstraction of the supernatant. The optical density of the solution was measured at 570 nm using a Mindray-96A microplate reader. The median inhibitory concentration (IC_{50}) was determined [11]. Therapeutic Index (TI) is the ratio of the highest cytotoxic concentration of the therapeutic extract to the inhibitory concentration of the extract that causes the desired efficacy. TI was calculated according to the formula:

TI = median inhibitory concentration / minimum inhibitory concentration

Animal experiments

Ethical clearance (no. UZREC 171110-030-RA-Level 02-PGM 2014/139) was obtained from the Research Animal Ethics Committee (RAEC) of the University of Zululand. The experiments were carried out in accordance with Directive 2010/63/EU on the use of laboratory animals [12]. The twelve white *Sprague-Dawley* rats (150 – 260 g) of both sexes were collected from the animal house in the Department of Biochemistry and Microbiology. Prior to the determination of the antidiarrhoeal and antimotility activities, rats were fed with standard food and given free access to water for one week to adapt to the laboratory conditions (temperature 23 ± 2 °C and 12 h light dark cycle). The rats were then fasted for 18 hours before the start of the experiment, but allowed free access to water.

Determination of antidiarrheal and antimotility activities

The method used for determination of antidiarrhoeal and antimotility activities was adopted from [13]. The experimental rats were divided into three groups of four rats each namely: Group A, Group B and Group C. Group A served as a negative control. It received vehicle distilled water (2 ml/kg). Group C served as a positive control. It received atropine at the dose of 5 mg/kg. Group B received fruit-pulp extracts (400 mg/kg). Each rat was put in its own cage. Diarrhoea was introduced to each rat by orally administering 0.2 ml of castor oil. After 30 minutes of administration of castor-oil, observation of the defecation was done for 5 hours. The onset time of faeces and number of normal and wet faeces were the determined parameters. The rats were then administered with 2 ml of charcoal meal (3 % deactivated charcoal in distilled water) orally. They were sacrificed 30 min thereafter in order to determine gastrointestinal motility. The intestinal distance moved by the charcoal meal from pylorus to caecum was measured and expressed as a percentage of distance travelled from pylorus to caecum. The mean movement of charcoal meal (Mc) was evaluated as the ratio of the distance travelled (Ld) by the charcoal meal to the intestinal length (Ls), expressed as a percentage (Eq 1), while inhibition of motility (Am), i.e., antimotility activity, was computed as in Eq 2.

$$Mc\ (\%) = (Ld/Ls)100 \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (1)$$

$$Am\ (\%) = \{Dl - (Ld/Dl)\}100 \quad \ldots\ldots\ldots\ldots \quad (2)$$

Statistical analysis

Data are presented as mean ± standard error mean (SEM, n = 4). Differences between mean values for experimental groups were calculated using Microsoft Excel 2010 while Origin 6.0 was used to compute IC_{50}. Data were analysed by one-way analysis of variance (ANOVA). $P \leq 0.05$ was regarded as significant and $p \leq 0.01$ as very significant.

RESULTS

Phytochemical profile of extract

Phytochemical screening of methanol extract of *S. cordatum* revealed the presence of alkaloids, cardiac glycosides, flavonoids, saponins and terpenoids. The total phenolic content was 16.4 ± 1.8 µg/mg, expressed as mg gallic acid/g of dry plant material.

Antibacterial activity

The extract showed the lowest MIC value of 3.13 mg/ml against *S. aureus (ATCC 25925), B. cereus (ATCC 10102), E. hirae* (ATCC 8043), *K. pneumonia* (ATCC 4352) and *P. aeruginosa (ATCC 7700)* and had the lowest MBC value of 3.13 mg/ml against *E. hirae* (ATCC 8043) (Table 1).

Cytotoxicity assay of the extract

The extract exhibited a concentration dependent cytotoxic effect on Caco-2 cells. The inhibitory concentration required for 50 % cytotoxicity (IC_{50}) was 92 µg/ml (Figure 1).

Therapeutic index

Therapeutic index of the fruit pulp extract was obtained on the selected bacterial species. It ranges from 0.1 - 0.3 (Table 2).

Antidiarrheal and antimotility activities of extract

Fruit pulp extract reduced the number of wet stools, total number of stools and onset time generally in comparison to the negative control (distilled water). Fruit pulp extract, in a dose-

Table 1: MIC and MBC (mg/ml) of *S. cordatum*

Bacterial strain	Pulp extract		Ciprofloxacin	
	MIC	MBC	MIC	MBC
S. aureus (ATCC 25925)	3.13	6.25	3.13	6.25
E.coli (ATCC 25922)	6.25	6.25	3.13	6.25
V. vulnificus (AL 042)	6.25	12.5	1.56	12.5
B. cereus (ATCC 10102)	3.13	6.25	3.13	3.13
S.typhimurium (ATCC70003)	6.25	6.25	1.56	6.25
E. hirae (ATCC 8043)	3.13	3.13	1.56	3.13
P. aeruginosa (ATCC 7700)	3.13	6.25	3.13	12.5
K. pneumonia (ATCC 4352)	3.13	12.5	3.13	12.5
V. fluvialis (AL 019)	6.25	12.5	1.56	12.5

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

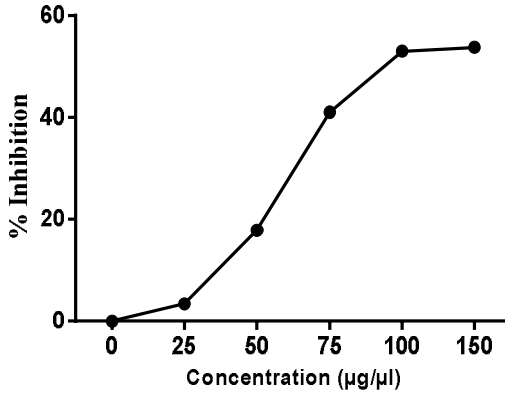

Figure 1: Inhibition of human colon cells treated with *S. cordatum* fruit-pulp extract

related manner (400 mg/kg of rat), exerted the antidiarrhoeal property by reducing intestinal motility as well. The results are displayed in Table 3 and 4.

DISCUSSION

Fruits are sources of chemical compounds that have been rarely used as alternative medicines to conventional therapy. Phytochemicals are naturally occurring and biologically active compounds that are chemically derived from plants. They possess strong antimicrobial, antidiarrheal and gastroprotective properties. The detected phytoconstituent in *S. cordatum* pulp extract implied that the pulps are potential sources for novel lead substances with potential therapeutic and preventive applications against GIT infections.

Table 2: Therapeutic index (TI) of *S. cordatum* fruit pulp extracts

Bacterial strain	TI (µg/ml)
S. aureus (ATCC 25925)	0.3
E. coli (ATCC 25922)	0.1
V. vulnificus (AL 042)	0.1
B. cereus (ATCC 10102)	0.3
S. typhimurium (ATCC70003)	0.1
E. hirae (ATCC 8043)	0.3
P. aeruginosa (ATCC 7700)	0.3
K. pneumonia (ATCC 4352)	0.3
V. fluvialis (AL 019)	0.1

Table 3: Effect of methanol extract of *S. cordatum* on castor oil (BV 394 Act/Wet 101/1965) induced rats

Group	Treatment	Dose	Onset time (min)	Nature of stool	
				Normal	Wet
A	Distilled water + Co	2 ml/kg	51	13±0.15	10.3±0.2
B	PE + Co	400 mg/kg	98	5.25±0.2	1.5±0.4
C	Atropine + Co	5 mg/kg	127	1.25±0.2	-

PE denotes fruit-pulp extract; Co denotes castor oil. Data are mean ± SEM (n = 4)

Table 4: Antimotility activity of methanol extracts of *S. cordatum* on castor oil (BV 394 Act/Wet 101/1965) induced rats

Group	Treatment	Dose	Mean total length of small intestine	Mean distance travelled by charcoal	Inhibition (%)
A	Distilled Water	2 ml/kg	130.8±3.97	106.8±6.54	-
B	PE + Co	400 mg/kg	114.8±3.68	59±3.34	49
C	Atropine	5 mg/kg	115.5±6.28	41.3±3.97	64

PE denotes fruit-pulp extract; Co denotes castor oil. Mean ± SEM (n = 4)

Ciprofloxacin is a broad-spectrum antibiotic which is effective against Gram-negative and Gram-positive bacterial strains [14]. It is widely used for treatment of urinary and respiratory infections as well as gastroenteritis. Ciprofloxacin had the inhibitory effects on all the selected bacterial strains with the lowest MIC (1.56 mg/ml) on *V. vulnificus* (AL 042), *V. fluvialis* (AL 019) and S. *typhimurium* (ATCC 700030). The highest MIC value (3.13 mg/ml) of ciprofloxacin was observed on all other selected bacterial strains.

Many naturally occurring compounds found in pulp extract possess antimicrobial activities. *S. cordatum* pulp extract showed broad-spectrum of antibacterial activity with the lowest MIC value of 3.13 mg/ml on *S. aureus* (ATCC 25925), *B. cereus* (ATCC 10102), *E. hirae* (ATCC 8043), *P. aeruginosa* (ATCC 7700) and *K. pneumonia* (ATCC 4352). Even though the antibacterial activity of the extract was more pronounced on all Gram-positive bacterial strains, it also showed remarkable antibacterial activity against Gram-negative bacteria (*P. aeruginosa* (ATCC 7700) and *K. pneumonia* (ATCC 4352) as well (see Table 1). Gram-negative bacteria, in addition to a

thin peptidoglycan layer (2 to 7 nm); have about 7 to 8 nm of the outer membrane. This outer membrane consists of an additional protective lipopolysaccharide layer that exhibits toxicity and antigenicity against antimicrobials [15]. It was thus concluded that the high resistance shown by some Gram-negative bacteria as compared to Gram-positive bacteria was due to the mechanism of the action by this layer. Gram-positive bacteria do not possess this layer and therefore they were generally sensitive to the action of the antibacterial phytochemicals detected in the extract. Gram-positive bacteria allowed the direct contact of the extract constituents with the phospholipid bilayer of the cell membrane of bacterial strains and enabled the extract to inhibit bacterial growth easily. The good potency of methanolic fruit extract has the MIC value ranging between 3.125 to 12.5 mg/ml [16]. The low MIC values displayed by the pulp extract implied that pulps have a potential to be used as sources of novel antibacterial agents.

Antimicrobial substances are considered as bactericidal agents when the ratio of MBC/MIC is $x \leq 4$ and bacteriostatic agents when the ratio of MBC/MIC is $x > 4$ [17]. The extract exhibited bactericidal effect on all selected bacterial species. However, the standard drug-ciprofloxacin showed bactericidal effect on all selected bacterial species with the exception on *V. fluvialis* (AL 019) and *V. vulnificus* (AL 042) where it showed the bacteriostatic effect.

Toxic phytochemicals can impose drastic damage to the immune cells` function, cause cell lysis, increase membrane permeability, hyper generation of reactive oxygen species and cell death. The higher the concentration of the extract, the more potential toxicity the extract revealed. The inhibitory concentration required for 50 % cytotoxicity (IC_{50}) was 92 µg/ml. The toxicity threshold level for plant extracts is considered highly toxic when $IC_{50} < 10$ µg/ml and moderate when $IC_{50} < 10 - 20$ µg/ml [18]. In addition to the cytotoxicity assay, the therapeutic index (Table 2) demonstrated safety-efficacy profile of the extract. To reach the toxic threshold of the extract, higher doses would have to be taken, thus, the extract is safe for consumption especially at lower concentrations.

The diarrheal activity of castor oil is attributed to its active cathartic glyceride known as ricinoleic acid. The ricinoleic acid stimulates intestinal hypersecretion, hypermotility and decreases gastrointestinal transit time [19]. Atropine is a tertiary amine belladonna alkaloid. It exerts its pharmacodynamic effect by binding competitively at the muscarinic receptors to prevent acetylcholine to bind. Atropine action results in reduction of excessive secretions of fluids and electrolytes, the intestinal hypertonicity and hypermotility [20]. The extract exhibited the antidiarrheal activity by reducing the number of wet stools, total stools, onset time and GIT motility in comparison to the negative control (distilled water).

Phytochemicals mediate antidiarrheal activity through antisecretory and antimotility mechanisms [21]. It was therefore esteemed that the antidiarrheal and antimotility activities revealed by the extract was due to the presence of the detected phytochemicals. The extract mechanism of action in antidiarrheal activity was esteemed to mimic that of an atropine. The results scientifically support *S. cordatum* pulp extract as potential source for effective and novel antidiarrheal agent.

CONCLUSION

S. cordatum fruit pulp extract has antibacterial, antidiarrheal and antimotility activities that can potentially prevent and treat GIT infections. These effects are likely due to the pharmacodynamic properties of the compounds in the extract. These effects are likely due to the phytochemical compounds in the extract. There is thus a need to isolate and investigate the activities of the compounds against GIT infections *in vivo*.

DECLARATIONS

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Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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