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# Evaluation of the antibacterial activity of *Syzygium cordatum* fruit-pulp and seed extracts against bacterial strains implicated in gastrointestinal tract infections

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**Gastrointestinal tract (GIT) infections are the major cause of high morbidity and mortality rates, especially in the developing countries. Fruit and seed extracts possess phytochemicals that are active against bacterial strains implicated in GIT infections. Different parts of *Syzygium cordatum* trees have been investigated pharmacologically against GIT infections previously with the exception of the fruits and seeds. This study aimed at evaluating the antibacterial activity of *S. cordatum* fruits and seeds against bacteria causing GIT infections. The harvested fruits were separated into fruit-pulp and seeds, dried and extracted with methanol using Soxhlet extraction. The extracts were phytochemically screened and micro dilution assay was used to evaluate antibacterial activity of the fruit-pulp and seed extracts against the selected GIT infecting bacteria. The crude extracts of fruit-pulp and seed exhibited the percentage yield of 10 and 6, respectively. The extracts showed the presence of phytochemicals with the total phenolic content of 21.4±1.4 µg/ml for seed extract and 16.4±1.8 µg/ml for fruit-pulp extract. Antimicrobial activity of the pulp extract exhibited the lowest minimum inhibitory concentration (MIC) of 3.13 mg/ml against *Bacillus cereus* (ATCC 10102), *Staphylococcus aureus* (ATCC 25925), *Klebsiella pneumoniae* (ATCC 4352), *Pseudomonas aeruginosa* (ATCC 7700), *Enterococcus hirae* (ATCC 8043) while the seed extract had an equal MIC value against *Klebsiella pneumoniae* (ATCC 4352). The antimicrobial activity was due to the detected phytochemicals and thus promoting *S. cordatum* fruits and seeds as potential new and cost effective sources for prevention and treatment of GIT infections.**

**Key words:** Gastrointestinal, fruits, seeds, phytochemicals.

## INTRODUCTION

Gastrointestinal tract (GIT) infections are the major cause of high morbidity and mortality rates, especially in the developing countries. Approximately more than 1.5 billion episodes of GIT infections that result in more than 3 million deaths are reported per year in the developing countries (WHO, 2009). The microbial resistance to most of the available drugs, the prohibitive costs of treatments consequent upon this resistance, the negative side

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**Abbreviations: GIT,** Gastrointestinal tract; **DMSO,** dimethyl sulfoxide; **MIC,** minimum inhibitory concentration; **MBC,** minimum bactericidal concentration.

effects of allopathic medicine and the newly emerging GIT infections have necessitated more research for novel, efficient, safe, and cost effective therapeutic compounds for the prevention and treatment of GIT infectious (El-Mahmood and Doughari, 2008; WHO, 2014). Plants extracts have served as good sources of antimicrobial agents against various pathogenic microorganisms implicated in GIT infections (Pradeep et al., 2008). The pharmacological activity of plant extracts against microbial strains causing GIT infections depends on the presence and concentrations of phytochemicals (Arup et al., 2012). According to Neethirajan et al. (2012), phytochemicals have strong antimicrobial and gastro-protective properties. Apart from the proven antimicrobial efficacy attributed to phytochemicals, plant based antimicrobial compounds possess the margin of safety without toxic side effects and also little chance of development of resistance to microorganisms (Fennell et al., 2004; El-Mahmood and Doughari, 2008; Ahmed et al., 2013; Gakunga et al., 2013). Although, fruits and seeds are excellent sources of therapeutic phytochemicals, fruits and seeds have been rarely used as medicine (van Wyk et al., 2009; Kossah et al., 2011; Srividhya et al., 2013). However, the increasing interest in novel sources for medicine against GIT infections has elevated more research for new therapeutic compounds from fruits and seeds especially from the wild edible plant species.

*Syzygium cordatum* are myrtaceous, edible trees native to the Republic of South Africa (RSA). *S. cordatum* trees are widely distributed in the Eastern Cape, KwaZulu-Natal and across northern part of RSA (Musabayane et al., 2005; Orwa et al*.*, 2009). *S. cordatum* trees also grow in areas with moist soil and high rainfall. The fruiting season of *S. cordatum* trees is usually from winter to summer in the RSA (Orwa et al., 2009). The fruits are purple, ovoid and fleshy up to 2 cm with 2.8 cm thick seeds. *S. cordatum* fruits rank fourth (after *Sclerocarya birrea*, *Englerophytum magalismontanum* and *Strychnos pungens*) as a preferred delicious fruit among indigenous South Africans (De Lange et al., 2005). The fruits are often fermented to produce potent intoxicating beverages. Jam and jelly are also manufactured from *S. cordatum* fruits (Young and Fox, 1982). The bark and leaves extracts of *S. cordatum* are used for treatment of GIT infections (Sibandze et al., 2010; Amabeoku and Deliwe, 2013). However, the therapeutic value of the fruits and seeds of *S. cordatum* has not been reported. This study was aimed at the evaluation of the antibacterial activity of *S. cordatum* fruit-pulp and seed extracts against bacterial strains implicated in GIT infections.

## MATERIALS AND METHODS

### Selected bacterial strains

The 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).

### Plant materials

Fruits from *S. cordatum* were randomly harvested in summer from the trees at the main campus of the University of Zululand, KwaZulu-Natal, RSA. The seeds from *S. cordatum* were manually removed from the fruit-pulps (fleshy part). The fruit-pulps and seeds were air-dried at room temperature. The dried *S. cordatum* fruit-pulps and seeds were separately ground to a coarse powder using an electric grinder and filtered with a filter of mesh size 1.0 mm to increase the surface area for solvents during the extraction process. The grounded samples were stored at 4°C until required for use.

### Extraction

The ground *S. cordatum* fruit-pulp and seed samples (100 g each) were separately subjected to Soxhlet extraction using 400 ml of methanol (Univ.AR). The samples were put on a mechanical shaker at a speed of 200 rpm at 37°C for 12 h. Extractions were repeated three times for each sample. The third extractions were left for 24 h. The extracts obtained were filtered through Whatman filter paper and concentrated using a Bùchi rotary evaporator at 45°C. The yields of each extract were weighed and re-dissolved in 100 ml of 10% dimethyl sulfoxide (DMSO) to the volume concentration of 100 mg/ml. The extracts were stored at 4°C until they were to be used. The percentage yields from *S. cordatum* fruit-pulp and seed extracts were calculated using the formula below that was used by Shahid (2012).

### Phytochemical screening

The extracted crude *S. cordatum* fruit-pulp and seed extracts were phytochemically screened. Phytochemical screening was done for all the extracts using the methods of Harborne (1973).

### Betulinic acid -Thin-layer chromatography

An original line of 2 cm from the edge, across the plate was drawn. Betulinic acid was loaded on thin-layer chromatography plate as standard indicator followed by loading of methanol extracts of *S. cordatum* fruit-pulp and seed, respectively. The thin-layer chromatography plate was placed in a chromatography tank containing mixture of hexane and ethyl acetate in the ratio of 7:3, respectively, covering about 1 cm of the plate. The chromatography was allowed to proceed until the hexane-ethyl acetate reaches the top of the plate. At that point, the chromatogram was removed from the tank and dried using hot air dryer. The plate was viewed under ultra violet light at 354 nm. It was then sprayed with 5% sulphuric acid-methanol solution. The appearance of a pink colour indicated the presence of betulinic acid (Walker, 1984).

### Quantification analysis of total phenolic content

The total phenolic contents were determined by the Folin-Ciocalteau method according to Makkar et al. (1993). An aliquot (0.2 ml) of 500 µg/ml methanolic fruit-pulp and seed extracts were

**Table 1.** Minimum inhibitory concentration, minimum bactericidal concentration in mg/ml and total activity in ml of the *S. cordatum* PE and SE extracts on the selected bacterial strains.

| Bacterial strain | Pulp extract | | | Seed extract | | | Ciprofloxacin | |
|---|---|---|---|---|---|---|---|---|
| | MIC | MBC | TA | MIC | MBC | TA | MIC | MBC |
| *S. aureus* (ATCC 25925) | 3.13 | 6.25 | 31 | 6.25 | 12.5 | 16 | 3.13 | 6.25 |
| *E. coli* (ATCC 25922) | 6.25 | 6.25 | 16 | 12.5 | 12.5 | 8 | 3.13 | 6.25 |
| *V. vulnificus* (AL 042) | 6.25 | 12.5 | 16 | 25 | 50 | 4 | 1.56 | 12.5 |
| *B. cereus* (ATCC 10102) | 3.13 | 6.25 | 31 | 12.5 | 12.5 | 8 | 3.13 | 3.13 |
| *S. typhimurium* (ATCC 700030) | 6.25 | 6.25 | 16 | 12.5 | 25 | 4 | 1.56 | 6.25 |
| *E. hirae* (ATCC 8043) | 3.13 | 3.13 | 31 | 6.25 | 12.5 | 8 | 1.56 | 3.13 |
| *P. aeruginosa* (ATCC 7700) | 3.13 | 6.25 | 31 | 12.5 | 12.5 | 4 | 3.13 | 12.5 |
| *K. pneumonia* (ATCC 4352) | 3.13 | 12.5 | 31 | 3.13 | 12.5 | 31 | 3.13 | 12.5 |
| *V. fluvialis* (AL 019) | 6.25 | 12.5 | 16 | 25 | 50 | 4 | 1.56 | 12.5 |
| Average total activity (ml) | | | 24 | | | 10 | | |

MIC, Minimum inhibitory concentration (mg/ml); MBC, minimum bactericidal concentration (mg/ml); TA, total activity (ml); PE, fruit-pulp extract; SE, seed extract.

made up to 1.0 ml with distilled water, respectively. 0.5 ml of Folin-Ciocalteau reagent (1N) was added, followed by 2.5 ml of sodium carbonate solution (20%). The mixtures were mixed properly, and then incubated at room temperature for 40 min. The absorbance of the blue-colored complex formed was measured at 725 nm against the appropriate blank. The total phenollic content was determined from the standard curve of tannic acid and expressed as equivalents of tannic acid (µg/ml).

#### Antimicrobial activity

The selected bacteria were inoculated into nutrient broth and incubated at 37°C for overnight. Afterwards, 1 ml from each of the bacteria species was pipetted into 9 ml of fresh prepared nutrient broth in separate test tubes labelled with corresponding microorganism. The test tubes were then incubated at 37°C for overnight. After overnight incubation, absorbance of the selected 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 $1.5 \times 10^8$ CFU/ml (Qaralleh et al., 2012).

#### Minimum inhibitory concentration (MIC)

A serial microdilution method was adapted as described by Eloff (1998) and Qaralleh et al. (2012) to measure the minimal inhibitory concentration (MIC) of the fruit-pulp and seed extracts. The MIC is the lowest concentration of the extract required to inhibit microorganisms. 96-well plates were used to quantitatively determine the MIC of both extracts. The sterile nutrient broth (50 µl) was added to all the wells of the 96-well plate, and 50 µl of the extracts (50 mg/ml, in 10% DMSO) was poured in the wells in the first rows and mixed well on separate plates. The extracts mixtures (50 µl) were removed from all the wells in the row A to perform a 3-fold serial dilution down the columns, respectively. 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. 10% DMSO was used as negative control while ciprofloxacin (20 µg/ml) was used as a positive control. The plates were covered and incubated at 37°C for overnight. 0.2 mg/ml of *P*-iodonitrotetrazodium violet (INT) solution was used after the incubation period. 40 µL of 0.2 mg/ml INT solution were added to each well and incubated at 37°C for 30 min. A reddish colour which was the result of INT being reduced by the metabolic activity of microorganism to formazan indicated microbial activity. 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 were reported (Table 1).

#### Minimum bactericidal concentration (MBC)

For the determination of MBC, the agar dilution method was used. The MBC of the extracts was determined by removing a loop full of each culture medium from the wells that no bacterial growths were streaked on different sterile nutrient agar plates. The agar plates were incubated at 37°C for 12 h. The lowest concentrations of the *S. cordatum* fruit pulp and seed extracts that exhibited the complete killing of test microorganisms were considered as the MBC (Qaralleh et al., 2012).

#### Determination of total activity

The total activity was calculated using the formula:

$$\text{Total activity} = \frac{\text{extracted 1g (mg)}}{\text{MIC (mg/ml )}}$$

The unit was converted to ml and represented the degree to which the bioactive extracts in a gram of powdered sample could be dilute and still inhibited the bacterial growth of the selected strains (Bag et al., 2013).

## RESULTS AND DISCUSSION

### Bioactive compounds

The percentage yields obtained from *S. cordatum* fruit-

**Table 2.** Preliminary phytochemical screening of *S. cordatum* PE and SE extracts.

| Phytochemical | Test | Sample | Result |
|---|---|---|---|
| Alkaloid | Dragendorff's test: | PE | + |
| | | SE | ++ |
| | Mayer's test: | PE | + |
| | | SE | ++ |
| Flavonoids | Alkaline reagent: | PE | + |
| | | SE | - |
| Saponins | Frothing: | PE | ++ |
| | | SE | + |
| Cardiac glycosides | Sodium nitroprusside | PE | + |
| | | SE | + |
| Tannins | Ferric chloride | PE | + |
| | | SE | + |
| Phenols | Ferric chloride | PE | + |
| | | SE | ++ |
| Terpenoids | Salkwosk | PE | + |
| | | SE | + |
| Betulinic acid (BA) | Tlc: | PE | + |
| | | SE | + |

-, Absence; +, low concentration; ++, moderate concentration; +++, high concentration. TLC, Thin layer chromatography; PE, fruit-pulp sample; SE, denotes seed sample.

**Table 3.** Total phenolic content in 500µg/ml of crude *S. cordatum* PE and SE extracts.

| Assay | Expression of results | Concentration (mg/g original sample) ±SER |
|---|---|---|
| Total phenolic | TAE:PE | 16.4±1.8 |
| | SE | 21.4±1.4 |

TAE, Tannic acid equivalents; PE, fruit-pulp sample; SE, denotes seed sample.

pulp and seed extracts were 10 and 6, respectively, after extracted with methanol. The good percentage yield as showed by both extracts is important when the extracts are needed for their biological activities. The ability of methanol solvent to give better percentage yields is due to its polarity. The results of the phytochemical screening are presented in Table 2. The total phenolic contents of the fruit and seed samples were also measured (Table 3). Bioactive metabolites have been reported to possess strong antimicrobial, anticancer, anti-allergic and gastroprotective properties (Neethirajan et al., 2012). The phytochemicals detected in varying proportions in both extracts were phenolic compounds, alkaloids, cardiac glycosides, phytosterols, flavonoids, saponins, terpenoids and betulinic acid. Flavonoids were not qualitatively detected in the seed extract however other detected bioactive compounds could have been responsible for the antimicrobial activity observed in this study. Moreover, quantitative analysis showed that the *S. cordatum* seed crude extract contained a significantly higher content of total phenolic compounds (21.4±1.4 µg/ml) as compared to *S. cordatum* fruit-pulp extract (16.4±1 µg/ml). This means that the seed extract may possess antibacterial and antidiarrheal activity even though flavonoids were not detected qualitatively. The detected phytoconstituent support the scientific idea that indigenous, traditional

fruits like *S. cordatum* fruits show potential sources of novel lead substances with potential therapeutic and preventive application against GIT infections.

### Antibacterial activity of *S. cordatum* fruit-pulp and seed extracts

Ciprofloxacin is a broad-spectrum antibiotic which is effective against Gram-negative and Gram-positive bacteria (Volans and Wiseman, 2010). Ciprofloxacin has bactericidal effect against *E. coli*, *Salmonella* spp., *Pseudomonas aeruginosa*, *Staphylococcus* spp. and *Klebsiella* spp. strains (Paw and Shulman, 2010). It is also active against *Streptococci spp*. Ciprofloxacin is widely used to treat urinary and respiratory infections as well as gastroenterities.

Ciprofloxacin (20 µg/ml) was used as a positive control on the tested bacteria in this study. Ciprofloxacin had inhibitory effects on all the test bacteria with the lowest MIC values of (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 recoded on all other selected bacterial strains. Many naturally occurring compounds found in fruit-pulp and seed extracts have been reported to possess antimicrobial activities. *S. cordatum* fruit-pulp extract showed broad-spectrum antibacterial action 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) while the seed extract had similar MIC value on *K. pneumoniae* (ATCC 4352).

Even though the antibacterial action of *S. cordatum* fruit-pulp extract was more pronounced on all Gram-positive bacterial strains, the extract did also show remarkable antimicrobial activities against some Gram-negative bacteria (*P. aeruginosa* (ATCC 7700) and *K. pneumonia* (ATCC 4352)) as well with the same MIC value of 3.13 mg/ml. The seed extract did also show the lowest MIC value of 3.13 mg/ml on the Gram-negative *K. pneumonia* (ATCC 4352).

Gram-negative bacteria, in addition to a thin peptidoglycan layer (2 to 7 nm), possess about 7 to 8 nm of the outer membrane. This outer membrane composes of additional protective lipopolyssachride layer that exhibits toxicity and antigenicity against antimicrobials or chemotherapeutic agents (Martinko and Madigan, 2006). It was concluded that the high resistance shown by some Gram-negative bacteria as compared to Gram-positive bacteria to both *S. cordatum* fruit-pulp and seed extracts was due to the mechanism of action of this layer. Gram-positive bacteria do not possess this layer and therefore they were highly sensitive to the action of antibacterial bioactive compounds found in both extracts. Gram-positive bacteria allow the direct contact of the extract constituents with the phospholipid bilayer of the cell membrane, enabling the antimicrobials to inhibit microorganism`s growth easily. The MIC values shown by *S. cordatum* fruit-pulp were found to be lower than those of *S. cordatum* seed extract generally. The low MIC values displayed by the fruit-pulp extract indicated a higher efficacy against bacteria causing GIT infections than the seed extract. The difference in efficacy of *S. cordatum* fruit-pulp and seed extracts may be due to the unique mechanism of action displayed by various phytochemicals (flavonoids) that were detected in the *S. cordatum* fruit-pulp extract but not in the *S. cordatum* seed extract, although the total phenolic content of seed extract (21.4±1.4 µg/ml) was higher than that of the *S. cordatum* fruit-pulp extract (16.4±1.8 µg/ml).

According to Jayashree et al. (2014), the good potency of methanolic fruit extract has MIC value ranging between 3.125 to 12.5 mg/ml. This implied that *S. cordatum* fruit-pulp and seed extracts have a potential as a source of novel antibacterial agents since they both possessed the MIC values ranging between 3.13 to 12.5 mg/ml against many bacterial strains used in this study. Antimicrobial substances can be considered as bactericidal agents when the ratio is MBC/MIC ≤ 4 and bacteriostatic agents when the ratio is MBC/MIC > 4 (Erhabor et al., 2013). Both *S. cordatum* fruit-pulp and seed extracts exhibited bactericidal effect on all selected bacterial species. However, the standard drug-ciprofloxacin showed bactericidal effect on all selected bacterial species except on *V. fluvialis* (AL 019) and *V. vulnificus* (AL 042) where it showed a bacteriostatic effect. The average total activity of fruit-pulp extract (24 ml) was much higher than that of a seed extract (10 ml). The obtained average total activities implied that, if a gram of extractable bioactive compounds present in one gram of ground *S. cordatum* fruit-pulp and seed was dissolved in 1 ml, it would still inhibit the bacterial growth of the selected strains (Bag et al., 2013). However, *S. cordatum* fruit-pulp would inhibit more bacteria than the seed extract.

## Conclusions

*S. cordatum* fruits and seeds possess antibacterial compounds that can be developed as new and cost-effective phytomedicine for therapy against GIT infections. Further studies will focus on the purification and identification of some of the bioactive compounds that are responsible for antibacterial activity.

## Conflict of interests

The author(s) did not declare any conflict of interest.

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