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The antibacterial and antidiarreal activities of the crude methanolic Syzygium cordatum [S.Ncik, 48 (UZ)] fruit pulp and seed extracts
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The antibacterial and antidiarrheal activities of the crude methanolic *Syzygium cordatum* [S.Ncik, 48 (UZ)] fruit pulp and seed extracts

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Diarrheal infections are the major cause of high morbidity and mortality rates, especially in the developing countries. Different parts (roots, bark and leaves) of the species *Syzygium cordatum* have been used as antidiarrheal extracts with the exception of its fruit-pulps and seeds. This study aimed at evaluating the antibacterial and antidiarrheal activity of *S. cordatum* pulp and seed extracts so to find newer and more cost-effective means to prevent diarrhoea. The harvested fruits were separated into pulp and seeds, dried and extracted with methanol using Soxhlet extraction. The extracts were screened for phytochemicals. The antibacterial and *in-vivo* antidiarrheal activities were determined using the microdillution method and castor oil-induced rat model, respectively. The percentage yields of 10 for fruit-pulp extract and 6 for seed extract were obtained. The detected phytochemicals were phenolics, alkaloids, cardiac glycosides, phytosterols, flavonoids, saponins, terpenoids and betulinic acid with the total phenolic content of 16.4±1.8 and 21.4±1.4 µg/mg for pulp and seed extracts, respectively. The pulp extract exhibited the lowest minimum inhibitory concentration (MIC) value of 3.13 mg/ml against some gram-positive and gram-negative bacteria while the seed extract had lowest MIC on. The *in vivo* antidiarrheal activity showed the percentage inhibition of 41 for the seed extract and 49 for pulp extract. The antibacterial and antidiarrheal activities were owed to the detected phytochemicals, and thus promoting *S. cordatum* fruit-pulps and seeds as potential sources of therapeutic compounds against diarrheal infections.

**Key words:** Antibacterial, antidiarrheal, antimotility, phytochemicals.

INTRODUCTION

Diarrheal infections are major causes of morbidity and mortality worldwide, especially in developing countries among infants and children. There are approximately 1.5 billion episodes of diarrheal infections per year. More than one in ten deaths of children under the age of 5 years are due to diarrhoeal infections (WHO and UNICEF, 2009). Diarrhoea is gastrointestinal disorder that is characterized by a decrease in the stool consistency and an increase in frequency, fluidity, or volume of the faeces during defecation for a period of...
days or weeks (Mazzolin et al., 2013). The most common symptoms of diarrhoeal infections range from mild and self-limiting symptoms (Mims et al., 2004).

However, severe diarrhoea may lead to the disordered gastrointestinal tract (GIT) motility, dehydration, electrolyte imbalance, acidosis and malnutrition (Dyer and Gould, 2011). Diarrhoea often occurs due to the damage of the intestinal mucosal cells by exotoxins and endotoxins of microbial origin in contaminated food and water and metabolic disorder in gastrointestinal tract (Kumar et al., 2001).

Several studies have reported the beneficial effects of fruit pulps and seed extracts in the treatment of diarrhoeal infections (Ashorobi and Umukoro, 2005; Maha et al., 2013). The antibacterial and anti diarrheal activit of fruit pulp and seed extracts depend on the presence and concentrations of phytochemicals (Arup et al., 2012). According to Neethiriajan et al. (2012), phytochemicals have strong antibacterial and antidiarrheal properties. Although fruits and seeds are excellent sources of therapeutic phytochemicals, pulps and seeds have been rarely used as medicine (van Wyk et al., 2009; Kossah et al., 2011; Srividhya et al., 2013).

However, the prohibitive costs and the negative side effects of allopathic medicine used against diarrheal infections have recently elevated fruits and seeds as sources for novel antidiarrheal agents. *S. cordatum* are edible fruit-trees native to the Republic of South Africa (RSA). They are widely distributed in the Eastern Cape, KwaZulu-Natal, across northern part of the RSA and in areas with high rainfall (Orwa et al., 2009). The bark and leaves of *Syzygium cordatum* trees have been proven to possess antidiarrhoeal properties (Sibandze et al., 2010; Amabeoku and Deliwe, 2013).

The fruits of *S. cordatum* have only been used for consumption and not for pharmacological purposes such as treatment of diarrheal infections. The fruits are purple, ovoid and fleshy up to 2 cm fruits with 2.8 cm thick seeds (Downs and Wilson, 2012). The fruiting season is usually from October to June in Republic of South Africa (RSA) (Drummond and Moll, 2002).

The study was undertaken to evaluate the antibacterial and antidiarrheal activities of *S. cordatum* pulp and seed extracts as to find novel sources that can be developed for treatment of diarrhoeal infections.

**MATERIALS AND METHODS**

Fruits of *S. cordatum* were randomly harvested in summer (February, 2014) from the trees at the main campus of the University of Zululand (UZ), KwaZulu-Natal, RSA. The fruits were washed with distilled water, seeds and pulps were manually separated. 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 form 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**

A Soxhlet extraction was done according to Bii et al. (2009) with some modifications. The ground *S. cordatum* fruit-pulp sample (100 g) was subjected to Soxhlet extraction using 400 ml of methanol (Univ.AR). The sample was put on a mechanical shaker at a speed of 200 rpm at 37°C for 12 h. The extract obtained was filtered through Whatman filter paper and concentrated using a Büchi rotary evaporator at 45°C. The yield of the extract was 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 yield from *S. cordatum* fruit-pulp extract was calculated using the formula below that was used by Shahid (2012).

\[
\text{% Yield} = \frac{\text{Weight of the extract (g)}}{\text{Weight of powdered sample (g)}} \times 100
\]

**Phytochemical compounds**

**Phytochemical screening**

The extracted crude *S. cordatum* fruit pulp and seed extracts were screened for phenolics, alkaloids, flavonoids, tannins, phenols, terpenoids, cardiac glycoside, saponins and betulinic acid. The phytochemical screening was done in all the extracts (except for betulinic acid) 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 extract of *S. cordatum* fruit-pulp. 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 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 phenolic content was determined from the standard curve of tannic acid and expressed as equivalents of tannic acid (μg/mg).

**Antimicrobial activity**

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), etc.
**Escherichia coli** (ATCC 25922), **Pseudomonas aeruginosa** (ATCC 7700) and **Vibrio vulnificus** (AL 042).

**Revival of the selected bacterial strains**

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 x colony forming unit (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 MIC of the fruit-pulp extract. The MIC is the lowest concentration of the extract required to inhibit microbial growth. 96-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 microplate 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 extract mixture (50 μl) were removed from all the wells in the 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. 10% DMSO was used as negative control while ciprofloxacin (20 μg/ml) was used as a positive control. The plate was 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 was 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 to be the indication of the absence of bacterial activity since the INT was not broken-down to form formazan. The test was replicated three times and the mean value was reported.

**Minimum bactericidal concentration (MBC)**

For the determination of MBC, the agar dilution method was used. The MBC of the extract was determined by removing a loop full of each culture medium from the wells that had no bacterial growths. 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 S. cordatum fruit-pulp extract that exhibited the complete killing of test microorganisms was considered as the MBC (Qaralleh et al., 2012).

**Antidiarrheal and antimotility activities**

**Animals**

Ethical clearance for the use of animals was collected from the Research Animal Ethics Committee (RAEC) of the UZ and the twelve white **Sprague-Dawley** rats (150 to 260 g) were collected from the animal house in the Department of Biochemistry and Microbiology at the same institution. 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 h before the start of the experiment to empty the GIT and to increase their responsiveness to the extracts and drugs used, but allowed free access to water (Orhan et al., 2013).

**Antidiarrheal activity**

The method used in for determination of antidiarrheal activity was adopted from Teke et al. (2007), with some modifications. The rats were divided into four groups of four rats each namely: Group A, Group B, Group C and Group D. Group A served as a negative control. It received vehicle distilled water (2 ml/kg) orally. Group D served as a positive control. It received atropine at the dose of 5 mg/kg orally by gavage. Group B and Group C received the seed and fruit-pulp extracts (400 mg/kg), respectively. 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 min of administration of castor-oil, observation of the defection was done for 5 h. The onset time of faeces and number of normal and wet faeces were the determined parameters. A score based on stool consistency was assigned as follows; normal-stool = A and wet-stools = B. The presence of normal stools was recorded as a positive result, indicating protection offered by the controls and the extracts from diarrhea while the presence of watery stools was recorded as negative results.

**Antimotility activity**

The method used in the antimotility test was adopted from Teke et al. (2007), with some modifications. The animals were divided into four groups of four rats each namely: Group A, Group B, Group C and Group D. Diarrhoea was introduced to each rat in all groups by orally administering 0.2 ml of castor oil. After 30 min of administration of castor-oil, all rats received different treatments. Group A served as a negative control and received distilled water (2 ml/kg) orally. Group B served as a positive control. It received atropine at the dose of 5 mg/kg orally by gavage. Group B and C received the seed extract and fruit-pulp extract of 400 mg/kg, respectively. Thereafter, each rat was put in its own cage after the administration of 2 ml of charcoal meal (3 % deactivated charcoal in distilled water) orally. The rats were sacrificed 30 min thereafter for determination of 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 in ratio to the intestinal length and percentage of inhibition were arithmetically measured. The following formulas were used:

\[
\text{% travelled} = \frac{\text{Length travelled by the charcoal meal}}{\text{Total length of small intestine}} \times 100
\]

\[
\text{% inhibition} = \frac{\text{Mean length of the duodenum} - \text{length of charcoal meal}}{\text{Mean length of duodenum}} \times 100
\]

**RESULTS**

The use of methanol as an extracting solvent resulted in
Table 1. Preliminary phytochemical screening of *S. cordatum* PE and SE samples and extracts.

<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th>Tests</th>
<th>Samples</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloid</td>
<td>Dragendorff's Mayer's</td>
<td>PE</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SE</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PE</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SE</td>
<td>++</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>Alkaline reagent</td>
<td>PE</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SE</td>
<td>-</td>
</tr>
<tr>
<td>Saponins</td>
<td>Frothing</td>
<td>PE</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SE</td>
<td>+</td>
</tr>
<tr>
<td>Cardiac glycosides</td>
<td>Sodium nitroprusside</td>
<td>PE</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SE</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>Ferric chloride</td>
<td>PE</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SE</td>
<td>+</td>
</tr>
<tr>
<td>Phenols</td>
<td>Ferric chloride</td>
<td>PE</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SE</td>
<td></td>
</tr>
<tr>
<td>Terpenoids</td>
<td>Salkwosk</td>
<td>PE</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SE</td>
<td>+</td>
</tr>
<tr>
<td>Betulinic acid (BA)</td>
<td>TLC</td>
<td>PE</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SE</td>
<td>+</td>
</tr>
</tbody>
</table>

Key: - denotes absence, + denotes low concentration, ++ denotes moderate concentration, +++ denotes high concentration, TLC denotes Thin layer chromatography and PE denote fruit-pulp extract.

a good percentage yield of 10 for fruit-pulp extract and 6 for seed extract. Phytochemicals are non-nutritional bioactive chemicals from plants that help plants to survive biotic and abiotic environmental changes and have therapeutic properties in humans. The total phenolic content of 16.4±1.8 and 21.4±1.4 µg/mg were obtained in pulp and seed extracts, respectively. The qualitative and quantitative analysis of phytochemicals from *S. cordatum* fruit-pulp and seed samples and extracts are presented in Table 1.

The antibacterial results are as presented in Table 3. Pulp extract showed the lowest MIC value of 3.13 mg/ml on *S. aureus* (ATCC 25925), *B. cereus* (ATCC 10102), *E. hirae* (ATCC 8043) and *P. aeruginosa* (ATCC 7700) isolates while the seed extract had the lowest MIC value (6.25 mg/ml) on all gram-positive bacteria. *S. cordatum* fruit-pulp and seed extracts exhibited different percentage of inhibition against the diarrheal activity in castor oil induced-rats. *S. cordatum* fruit-pulp and seed extracts both reduced the number of wet stools, total stools and onset time generally in comparison to the negative control (distilled water). *S. cordatum* fruit-pulp and seed extracts, in a dose-related manner (400 mg/kg of rat), exerted the antidiarrhoeal properties by reducing intestinal motility. The results are tabled in Tables 4 and 5 below.

**DISCUSSION**

The use of methanol as an extracting solvent resulted in a good percentage yield of 10 for fruit-pulp extract and 6 for seed extract. The good percentage yields implied that methanol is an important solvent to be used when determining the biological activities of the extracts. The ability of methanol solvent to extract good yields is owed to its polarity.

Phytochemicals have been reported to possess strong antibacterial, antidiarrheal and gastroprotective properties (Neethirajan et al., 2012). The phytochemicals detected
Table 2. Total phenolic content in 500 µg/ml of crude methanolic S. cordatum pulp and seed extract.

<table>
<thead>
<tr>
<th>Assay Expression of results</th>
<th>Concentration (µg/mg original sample) ± SER</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total phenolic</td>
<td>TAE: Pulp-extract, seed extract</td>
</tr>
</tbody>
</table>

Values are the average of duplicates experiments and represented as mean ± standard error (SER) and were expressed as µg/mg – where TAE denotes tannic acid equivalent.

Table 3. MIC and MBC (mg/ml) of the S. cordatum pulp and seed extracts on the selected bacterial strains known to cause GIT infections.

<table>
<thead>
<tr>
<th>Bacterial strains</th>
<th>Pulp extract</th>
<th>Seed extract</th>
<th>Ciprofloxacin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MIC</td>
<td>MBC</td>
<td>MIC</td>
</tr>
<tr>
<td>S. aureus (ATCC 25925)</td>
<td>3.13</td>
<td>6.25</td>
<td>6.25</td>
</tr>
<tr>
<td>E.coli (ATCC 25922)</td>
<td>6.25</td>
<td>6.25</td>
<td>12.5</td>
</tr>
<tr>
<td>V. vulnificus (AL 042)</td>
<td>6.25</td>
<td>12.5</td>
<td>25</td>
</tr>
<tr>
<td>B. cereus (ATCC 10102)</td>
<td>3.13</td>
<td>6.25</td>
<td>12.5</td>
</tr>
<tr>
<td>E. hirae (ATCC 8043)</td>
<td>3.13</td>
<td>13.13</td>
<td>6.25</td>
</tr>
<tr>
<td>P. aeruginosa (ATCC 7700)</td>
<td>3.13</td>
<td>6.25</td>
<td>12.5</td>
</tr>
</tbody>
</table>

Table 4. Effects of the crude methanolic S. cordatum PE and SE extracts on castor oil-induced rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatments</th>
<th>Doses</th>
<th>Onset times (min)</th>
<th>Stools</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Distilled water plus Co</td>
<td>2 ml/kg</td>
<td>51</td>
<td>normal, 13±0.15, 10.3±0.21</td>
</tr>
<tr>
<td>B</td>
<td>SE plus Co</td>
<td>400 mg/kg</td>
<td>68</td>
<td>8.25±0.17, 2.75±0.31</td>
</tr>
<tr>
<td>C</td>
<td>PE plus Co</td>
<td>400 mg/kg</td>
<td>98</td>
<td>5.25±0.20, 1.5±0.41</td>
</tr>
<tr>
<td>D</td>
<td>Atropine plus Co</td>
<td>5 mg/kg</td>
<td>127</td>
<td>1.25±0.19, 0</td>
</tr>
</tbody>
</table>

Key: Values are represented as mean ± standard error. PE denotes fruit-pulp extract, SE denotes seed extract and Co denotes castor oil.

in both extracts were phenolic compounds, alkaloids, cardiac glycosides, phytosterols, flavonoids, saponins, terpenoids and betulinic acid (Table 1). The quantitative analysis showed the significant amount of the total phenolic compounds (16.4±1 µg/mg) in pulp extract and (21.4±1.4 µg/mg) in seeds extract (Table 2). The detected phytoconstituent implied that S. cordatum pulps and seeds can be potential sources for novel lead substances with therapeutic and preventive applications against bacteria that may cause diarrheal infections.

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, Streptococcus pp and Klebsiella spp.strains (Paw and Shulman, 2010). Ciprofloxacin is widely used to treat urinary and respiratory infections as well as gastroenteritis. Ciprofloxacin (20 µg/ml) was used as a positive control on the tested bacteria in this study. Ciprofloxacin had the inhibitory effects on all the selected 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 pulp extract have been reported to possess antibacterial activity. S. cordatum 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) and P. aeruginosa (ATCC 7700). Even though the antibacterial action of S. cordatum pulp extract was more pronounced on all gram-positive bacterial strains, the extract also show remarkable antibacterial activity against gram-negative bacteria (P. aeruginosa (ATCC 7700) as well with the same MIC value of 3.13 mg/ml. 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 lipopolysachride layer that exhibits toxicity and
antigenicity against antimicrobials or chemotherapeutic agents (Martinco 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 pulp extract was due to the mechanism of action of this layer. Gram-positive bacteria do not possess this layer and therefore, they were generally sensitive to the action of the antibacterial action of the detected phytochemicals. Gram-positive bacteria allow the direct contact of the extract constituents with the phospholipid bilayer of the cell membrane, enabling the antibacterial compounds to inhibit bacterial growth easily.

The low MIC values displayed by the fruit-pulp extract indicated its higher efficacy against bacteria causing GIT infections than the seed extract. According to Jayashree et al. (2014), the good and promising potency of methanolic fruit extract has the MIC value ranging between 3.125 to 12.5 mg/ml. This implied that S. cordatum pulp extract has a potential to be used as sources of novel antibacterial agent. Antimicrobial substances are 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). S. cordatum fruit-pulp 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. vulnificus (AL 042) where it showed the bacteriostatic effect.

Castor oil is an effective emollient laxative agent. Castor oil causes a decrease in fluid and nutrient absorption, increase in the electrolyte secretion and water and produces alterations in intestinal motility (Priff and Harold, 2005). The diarrheal activity of castor oil is attributed to its active cathartic glyceride known as ricinoleic acid (Chambers et al., 2015). Thus, castor oil-induced diarrhoea is as a result of the action of ricinoleic acid formed from the hydrolysis of its triglyceride in the duodenum by pancreatic lipase. The ricinoleic acid formed from the hydrolysis of its triglyceride in the duodenum by pancreatic lipase. The ricinoleic acid induced diarrhoea is as a result of the action of ricinoleic acid formed from the hydrolysis of its triglyceride in the duodenum by pancreatic lipase. The ricinoleic acid stimulates intestinal hypersecretion, hypermotility and decreases gastrointestinal transit time (Schellack, 2004).

In this study, castor oil was used to induce diarrhea in the test rats. Atropine was used in this study as a positive control in in-vivo antidiarrheal activity. Atropine is a tertiary amine belladonna alkaloid (Chambers et al., 2015). Atropine is a racemic hyoscyamine tropic acid ester of the base tropine. Atropine has high affinity for muscarinic receptors (Hollinger, 2008; Champe and Harvey, 2009). Atropine exerts its pharmacodynamic effect by binding competitively at the muscarinic receptors to prevent acetylcholine to bind, and thus reversing excessive secretions of fluids and electrolytes. Atropine actions reduce the intestinal hypertonicity and hypermotility of the GIT and thus act as an antidiarrheal agent (Lehne, 2004).

Group C was fed S. cordatum pulp extract at a dose of 400 mg/kg of a rat weight. Group C had stool onset time of 68 minutes, total normal stools of 8.25±0.17 and the total wet stools of 2.75±0.3 while Group B (seed extract) at the same dose as Group C and had the stool onset time of 98 minutes, total normal stool of 5.25±0.20 and the total wet stools of 1.5±0.41 in comparison to the Group A (distilled water) which had stool onset time of 51 min, total normal stools of 13±0.15 and the total wet stools of 10.3±0.2. Group D had the longest onset time (127 min) and the least total number of normal stools (1.25±0.19) with wet stools not being observed. S. cordatum fruit-pulp and seed extracts exhibited the antidiarrheal activity by reducing the number of wet stools, total stools and onset time generally. Phytochemicals mediate antidiarrheal activity through antisecretory and antimitotility mechanisms (Cowan, 2015). It was therefore esteemed that the antidiarrheal activity observed in Group B and C was due to the presence of these phytochemicals in S. cordatum pulp and seed extracts. The extracts mechanism of action in antidiarrheal activity was esteemed to mimic that of an atropine (control).

Group D was given an antimuscarinic drug (atropine) and had the highest inhibitory percentage of 64 followed by Group C with 49 of inhibition. Group B (seed extract) had the inhibitory percentage of 41. There was no inhibition observed in Group A (distilled water). The reduction in the distance travelled by charcoal in the S. cordatum fruit-pulp and seed extracts treated groups indicated that S. cordatum pulp and seed extracts exerted antidiarrheal activity by decreasing the GIT motility. The reduction of GIT motility of extracts in
comparison to the negative control (distilled water) was attributed to the presence of the detected phytochemicals (saponins, alkaloids, triterpenoids, flavonoids, tannins and betulinic acid). Phytochemicals exert similar mode of action as antimotility agents (Ahmad et al., 2006; Saleem et al., 2010; Chollet and Gleason, 2012).

Thus, *S. cordatum* pulp and seed extracts might have exhibited the antimotility action through the same mechanism of action exerted by the drug-atropine. The results scientifically support *S. cordatum* pulp and seed extracts as potential sources for effective, novel antibacterial and antidiarrheal agents.

Conclusion

*S. cordatum* pulp and seed extracts demonstrated the therapeutic and biological efficacy (antibacterial, antidiarrheal and antimotility activities). Due to the pharmacodynamic effects revealed by *S. cordatum* pulp and seed extracts, *S. cordatum* pulps and seeds can be viewed as satisfactorily beneficial sources of therapeutic compounds against diarrheal infections. Further studies will focus on the purification and identification of some of the bioactive compounds that are responsible for the antibacterial and antidiarrheal activities.

Conflict of Interest

The authors have not declared any conflict of interest.

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Shahid AA (2012). Biological activities of extracts and isolated compounds from *Bauhinia galpinii* (Fabaceae) and *Combretum vendeae* (Combretaceae) as potential antidiarrheal agents-1 (Doctoral dissertation, University of Pretoria).


In-vitro effects of almond oil, barks and leaves extracts of *Baillonella toxisperma* (Pierre) against *Trichophyton soudanense* and *Trichophyton rubrum*

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The purpose of this study was to evaluate anti-trichophytic effects of *Baillonella toxisperma* (Pierre) extracts against *Trichophyton rubrum* and *Trichophyton soudanense*. The tests were performed by the agar dilution method and the results have showed the effective inhibition of the mycelia growth of theses strains by *B. toxisperma* (Pierre) extracts. After 7 days of incubation at 28°C, the percentages of mycelia growth inhibition PI (%) by plant extracts and fluconazole were range between 20.81 and 100% on *T. soudanense*, and between 19.92 and 100% on *T. rubrum*. Some *B. toxisperma* (Pierre) extracts were exhibited similar anti-trichophyton activities compared to those obtained with fluconazole which was used like reference antifungal drug (p < 0.05). The best anti-trichophyton activities of *B. toxisperma* (Pierre) extracts were obtained at 5 mg/ml on *T. Rubrum* with almond oil and hexane phase of water-hexane partition of hydro-ethanol crude extract of barks HBb, and at 10 mg/ml on *T. soudanense* with bromobentseeni (HBb). This anti-trichophyton activity of *B. toxisperma* (Pierre) extracts was attributed to secondary metabolites. The phytochemical analysis has showed that the plant contain bioactive metabolites groups such as phenols, flavonoids, saponins, coumarin, tanins, anthocyanins, lipids and triterpenes. The metabolic groups have been mentioned in several studies for their antifungal activities. The results from this study provide a scientific validation for the ethno-medicinal uses of *B. toxisperma* (Pierre) in the treatment of fungal infections.

**Key words:** Trichophytic infections, *Baillonella toxisperma* (Pierre), bioactive metabolites, anti-trichophyton drugs.

INTRODUCTION

Trichophytons species are highly prevalent in adults and represents a serious clinical problem (Kwon et al., 2004). Among these species, *T. soudanense*, is a common causes of tineacapitis in parts of Africa (Menan et al., 2002) and *T. rubrum* is the most frequently isolated dermatophyte, accounting for 80 to 90% of the

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Author(s) agree that this article remain permanently open access under the terms of the Creative Commons Attribution License 4.0 International License
infectious strains (Elewski et al., 2002). These infectious agents are responsible to superficial fungal infections of the skin, nails and hair of humans and animals (Kobylak et al., 2015). These strains colonize the upper layers of dead skin and are the most common cause of athlete’s foot, jock itch and ringworm worldwide (Zaugg et al., 2009). The prevalence of trichophytic infections, due to T. soudanense and T. rubrum, has considerable increased all over the world especially in low and middle income countries like Cameroon. According to the development of fungal resistance and toxic side-effects problems correlated to the use of several current anti-trichophytic drugs, there is a need to search for news actives principles.

In the last few decades, the traditional system of medicine has become the interest of many scientists as a tool to improve the global health issues (Radfar et al., 2012). Several antifungal compounds isolated from medicinal plants have been reported in many studies for their capacities to inhibit the fungal growth. For example, the plants metabolites such as benzoic acid, thymol, citronelol, geraniol and 1,8-cineol have been reported by Shin and Lim (2004) for their interesting antifungal activities against T. rubrum, T. soudanense, T. schoenleinii and T. tonsurans. Lupeol, a significant lupine-triterpene represented in plant, has been reported by Gallo and Sarachine (2009) to have remarkable antifungal properties against yeasts and filamentous strains like Candida albicans, Cryptococcus neoformans, Microsporum canis and Aspergillus fumigates. Medicinal plants have been used for centuries as remedy for the treatment of various diseases and are the major sources of drugs (Nostro et al., 2000). Many bioactive molecules isolated in plants are in long time served to develop modern antifungal drugs.

The Cameroonian traditional medicine is a reservoir of several medicinal plants species used to cure a lot of infectious diseases. Among these plants, Baillouilla toxisperma (Pierre) is a Sapotaceae family plant very reputed traditionally for her antimicrobial potential. This plant is used to cure infectious diseases like fungal infections, diarrhea, sexually transmitted infections, rheumatism and toothache. Almond oil of this plant is used in divers by the inhabitants of Cameroon rural zones to cure skin affections like scabies, itch, dermatitis and many others superficial infections due to microbes. The antifungal activity of B. toxisperma (Pierre) was studied for the first time by Riwom et al. (2015) against five yeasts among C. albicans, Candida sp., Candida parasilopsis and Cryptococcus neoformans.

The results showed remarkable ability of B. toxisperma (Pierre) extracts to inhibit the in vitro growth of these organisms. The main objective of this study is to provide a scientific validation for the ethno-medicinal uses of B. toxisperma (Pierre) extracts in the treatment of infectious diseases.

This study evaluates anti-trichophyton activity of barks and leaves extracts of B. toxisperma (Pierre) against T. soudanense and T. rubrum.

MATERIALS AND METHODS

Fungal strains

Fungal material consist two filamentous strains, T. soudanense and T. rubrum. These fungal strains were gratefully given by “Centre Pasteur du Cameroun” and were conserved at +4°C on Sabouraud Dextrose Agar (SDA). Before biological tests, these strains have been first reactivated.

Plant material

The plant material was collected at Est-Cameroon region in Dimako on 21th October, 2011. The botanic identification was performed at National Herbarium of Cameroon where a specimen has been deposed on number 54060/HNC.

Extraction of almond oil

A weighed quantity of 300 g of dried almond of B. toxisperma (Pierre) was crushed into a mortar. Pastry obtained was carried at ebullition into a pot containing 250 ml of distilled water. The mixture was constantly moved until total evaporation of water and appearance of oil. After cooling at 25°C during 30 min, the cooked pastry was pressed to extract the oil. Oil thus obtained has been warmed on wood fire, decanted and filtered using a sieve to eliminate the lather and impurities (Bouopda, 2013). The pH of oil was determined at 25°C using a pH meter and the density has been determined using scales.

Extraction of barks and leaves

The barks and leaves of B. toxisperma (Pierre) were shade-dried and crushed using electric grinder. A weighed quantity (100 g) of the any powder was then subjected to extraction in water, ethanol, water-ethyl acetate mixture (3:7), methanol and ethyl acetate. After 3 days of maceration, extract was filtered through Whatman No 1 filter paper and evaporated using a rotary evaporator. Extractions were repeated tree times. Aliquots of 10 g of water/ethyl acetate powders were fractioned by liquid-liquid partition in 400 ml of water-hexane mixture (1:1) by emulsification. After decantation, the aqueous and hexane phases obtained where filtered and concentrated (Riwom et al., 2015).

Determination of extraction yield

The extraction yield EY (%) was determined for extraction of oil and for extraction of barks and leaves of B. toxisperma (Pierre) according the following formula:

\[
EY(\%) = \frac{(Mo/Me)}{EY} \times 100
\]

Mo: Mass of extract obtained (oil, barks or leaves extract)
Me: Mass of powder extracted (almonds, barks or leavespowder)

Phytochemical screening

The standard procedures described into literature by Harbone.
Table 1. Extraction yield and physicochemical properties of almond oil of *Baillonella toxisperma* (Pierre)

<table>
<thead>
<tr>
<th>Extraction of almond oil</th>
<th>Mass of almonds extracted</th>
<th>Volume of oil obtained</th>
<th>Mass of oil obtained</th>
<th>Extraction yield</th>
<th>Physicochemical properties of oil obtained</th>
</tr>
</thead>
<tbody>
<tr>
<td>Almond oil of <em>Baillonellatoxisperma</em> (Pierre)</td>
<td>300g</td>
<td>12.7 ml</td>
<td>10.78g</td>
<td>3.59%</td>
<td>pH : 6.98, Density (mg/mL) : 849 Color : Yellowish, Appearance at room temperature: fluid, Consistence : fatty at low temperature</td>
</tr>
</tbody>
</table>

(1998) and Edeoga et al. (2005) have been used for phytochemical tests.

**Evaluation of mycelial growth inhibition on agar medium**

A weighed quantity of 400 mg of powder of each extract or of reference antifungal (Fluconazol) was introduced into an Erlenmeyer flask containing initially 10 ml of Sabouraud Dextrose Agar (SDA) medium maintained in fusion at 45°C. After homogenization by vigorous agitation of the mixture, a double dilution of the tested substance was carried out. In fact, 5 ml of mixture previously prepared was deducted and introduced into Petri dishes of 55 cm of diameter, and the remaining solution in test tube (5 ml) was completed with 5 ml of sterile SDA medium in fusion, and after homogenization the operation was repeated to obtain the following concentrations. The obtained concentrations of vegetal extracts and Fluconazol in the SDA medium were ranged between 40 and 2.5 mg/ml. The Petri dishes were left at room temperature until solidification of different preparations. Then, a mycelium disk of 6 mm of diameter, deducted to pre-culture of 7 days of *T. soudanense* or *T. rubrum* was deposed in the center of each dishes. The positives (sterility of the medium controls) and the negatives (growth controls) controls were realized. The Petri dishes were sealed using a parafilm paper and incubated in an incubator at 28°C. After incubation, the mycelial growth diameters were measured using a caliper. Other test was performed in triplicate. Fungitoxicity was expressed in terms of percentage of mycelial growth inhibition [PI (%)] determined according the formula of Pander et al. (1982).

\[
PI(\%) = \left(\frac{DT - D}{DT}\right) \times 100
\]

DT: Diameter of mycelial growth of negative control

D: Diameter of mycelial growth of test

**Statistical analysis**

The statistical analysis was done using Graph Pad Prism 5. Other test was performed in triplicate. Data were reported as the mean ± standard deviation from replicate experiments and one-way analysis of variance (ANOVA) was applied via Dunnett's post hoc multiple comparison tests for determining the statistical significance between *B. toxisperma* (Pierre) extracts (Dependant groups) and Fluconazole (Control group). The values were considered significantly different in the level of *p < 0.05*. Microsoft Excel has been used to drawn diagrams.

**RESULTS**

**Extraction**

The yields of extraction and the physico-chemical characteristics of extracts obtained from almond, barks and leaves of *B. toxisperma* (Pierre) are mentioned in Tables 1, 2 and 3. These results show that, the extraction yields of barks range between 6.84 (aqueous extract) and 14.52% (methanol extract) respectively. The extraction yields of leaves are range between 5.44 (aqueous extract) and 16.76% (ethanol extract). Alcohol solvents have proved to be the best solvents for the extraction of barks and leaves crude extracts of *B. toxisperma* (Pierre). From the liquid-liquid partition of hydro-ethanol extracts in water-hexane system, the best extraction yields have been obtained with hexane phases, with a yield of 52.2% for hexane phase of hydro-ethanol extract of barks and 39.5% for hexane phases of hydro-ethanol extracts of leaves. Almond oil extraction has shown a yield of 3.59%.

**Phytochemical screening**

The qualitative detection of secondary metabolites groups in *B. toxisperma* (Pierre) extracts has been carried out according to the standard procedures described in literature. This screening released that *B. toxisperma* (Pierre) contains bioactive metabolites groups like phenols, flavonoids, saponins, coumarin, tanins, anthocyanins, lipids and triterpenes. The results obtained from phytochemical analysis of each extract are summarized in Table 4.

**Evaluation of the mycelia growth inhibition**

The mycelial growth inhibition has been evaluated on agar medium by agar dilution method. Diameters of mycelia growth inhibition of fungi strains on agar medium by *B. toxisperma* (Pierre) extracts and fluconazole after 7 days of incubation are ranged between 0.00±0.00 and 21.8±0.89 mm on *T. soudanense*, and between 0.00±0.00 and 22.2± 0.74 mmon *T. rubrum* (Tables 5 and 6). The percentage of mycelial growth inhibition [PI (%)] obtained from *B. toxisperma* (Pierre) extracts and
Table 2. Extraction yield and physicochemical properties of barks and leaves extracts of B. Toxisperma (Pierre).

<table>
<thead>
<tr>
<th>Extraction solvents</th>
<th>Part of vegetal extracted</th>
<th>Mass of vegetal powder extracted (g)</th>
<th>Mass of extract obtained (g)</th>
<th>Extraction yield (%)</th>
<th>Physicochemical properties extracts obtained</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>Barks</td>
<td>100</td>
<td>6.84</td>
<td>6.84</td>
<td>Brown Powdery</td>
</tr>
<tr>
<td></td>
<td>Leaves</td>
<td>100</td>
<td>5.44</td>
<td>5.44</td>
<td>Dark-green Powdery</td>
</tr>
<tr>
<td>Water/ethanol (3:7)</td>
<td>Barks</td>
<td>100</td>
<td>12.66</td>
<td>12.66</td>
<td>Brown Pasty</td>
</tr>
<tr>
<td></td>
<td>Leaves</td>
<td>100</td>
<td>12.58</td>
<td>12.58</td>
<td>Green Pasty</td>
</tr>
<tr>
<td>Ethanol</td>
<td>Barks</td>
<td>100</td>
<td>13.70</td>
<td>13.70</td>
<td>Brown Pasty</td>
</tr>
<tr>
<td></td>
<td>Leaves</td>
<td>100</td>
<td>16.28</td>
<td>16.28</td>
<td>Green Pasty</td>
</tr>
<tr>
<td>Methanol</td>
<td>Barks</td>
<td>100</td>
<td>14.52</td>
<td>14.52</td>
<td>Brown Powdery</td>
</tr>
<tr>
<td></td>
<td>Leaves</td>
<td>100</td>
<td>16.76</td>
<td>16.76</td>
<td>Dark-green Powdery</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>Barks</td>
<td>100</td>
<td>8.16</td>
<td>8.16</td>
<td>Brown Pasty</td>
</tr>
<tr>
<td></td>
<td>Leaves</td>
<td>100</td>
<td>6.34</td>
<td>6.34</td>
<td>Green Pasty</td>
</tr>
</tbody>
</table>

Table 3. Extraction yield and physicochemical properties of extracts obtained from water-hexane partition of hydro-ethanol crude extracts of barks and leaves B. toxisperma (Pierre).

<table>
<thead>
<tr>
<th>Extract partitioned in water-hexane mixture</th>
<th>Mass of extract (g)</th>
<th>Solvent phase</th>
<th>Yield (%)</th>
<th>Physicochemical properties extracts obtained</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydro-ethanol extract of barks</td>
<td>10</td>
<td>Water</td>
<td>16</td>
<td>Blackish Powdery</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hexane</td>
<td>52.2</td>
<td>Brown Fatty</td>
</tr>
<tr>
<td>Hydro-ethanol extract of leaves</td>
<td>10</td>
<td>Water</td>
<td>26</td>
<td>Green Powdery</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hexane</td>
<td>39.5</td>
<td>Green Fatty</td>
</tr>
</tbody>
</table>

Fluconazole are ranged between 20.81 and 100% on T. soudanense, and between 19.92 and 100% on T. rubrum. According to Adejumo and Bamidele (2009), a mycelial growth inhibition higher than 60% is considered effective, and an inhibition of 100% indicates no mycelial growth on agar plate (0.00±0.00 mm). The results (Figures 1 and 2) show that:

1. At 2.5 mg/ml, the mycelial growth inhibition was effective with HBb (60.82 %) and Fluconazole (72.48 %) on T. soudanense, and with almond oil (65.65 %) and HBb (64.66 %) on T. rubrum.
2. At 5.0 mg/ml, the mycelial growth inhibition was effective (60 < PI (%) < 100) with HBb (73.38 %), Lb (67.01 %) and HLb (68.10 %) on T. soudanense, and with extract Bb (73.31 %) on T. rubrum. A total inhibition (100 %) was observed on T. soudanense with HBb and fluconazole, and with almond oil, Bb, HBb and Fluconazole on T. rubrum.
3. At 10 mg/ml, the inhibition was effective (60< PI (%)< 100) on T. soudanense with Ba (60.53%), Bb (63.35%), Bc (61.07 %), Bd (68.50 %), and with Bc (61.07 %), Bd (70.27 %), Lb (65.53 %), HLb (68.08 %) on T. rubrum. A total inhibition (100 %) was observed on T. soudanense with HBb and fluconazole, and with almond oil, Bb, HBb and Fluconazole on T. rubrum.
4. At 20 mg/ml, the mycelial growth inhibition was effective (60 < PI (%) < 100) with Ba (68.24%), Be (60.46%), La (66.94%), WLb (73.42%), Lc (70.49%) and Le (72.59%) on T. soudanense, and with Ba (62.35%), WBB (63.78%), Bc (68.72%), Be (64.54%), Lb (71.87%), WLb (60.35%), Lc (67.49%), Ld (67.05%) and Le (60.35%) on T. rubrum. A total inhibition (100 %) was observed with almond oil, Bb, HBb, Bc, Bd, Lb, HLb and Fluconazole on T. soudanense, and with almond oil, Bb, HBb, Bc, Bd, Lb, HLb and Fluconazole on T. rubrum.
5. At 40 mg/ml, a total inhibition (100 %) was observed on T. soudanense with almond oil, Ba, Bb, HBb, Bc, Bd, Lb, WLb, HLb, Lc, Le and Fluconazole, and with almond oil, Bb, HBb, Bc, Bd, Lb, HLb and Fluconazole on T. rubrum. The inhibition was effective (60 < PI (%) < 100) with WBB (66.87%), Be (72.12%), La (73.93%) and Ld...
Table 4. Phytochemical screening.

<table>
<thead>
<tr>
<th>Metabolic groups</th>
<th>Baillonellatoxisperma (Pierre) extracts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Barks</td>
</tr>
<tr>
<td></td>
<td>Ba</td>
</tr>
<tr>
<td>Phenols</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
</tr>
<tr>
<td>Coumarins</td>
<td>+</td>
</tr>
<tr>
<td>Tanins</td>
<td>+</td>
</tr>
<tr>
<td>Anthocyanins</td>
<td>+</td>
</tr>
<tr>
<td>Lipids</td>
<td>-</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>-</td>
</tr>
<tr>
<td>Triterpenes</td>
<td>+</td>
</tr>
</tbody>
</table>

(65.60%) on *T. soudanense*, and with Ba (73.31%), Wbb (72.31%), Be (68.60%), La (64.78%), WLb (68.72%), Lc (72.07%), Ld (66.49%) and Le (72.03%) on *T. rubrum*.

**DISCUSSION**

The results obtained to the antifungal sensitivity (Tables 5 and 6) reveal that *B. toxisperma* (Pierre) extracts managed to inhibit the mycelium development of *T. soudanense* and *T. rubrum*. Almond oil and hexane phases of water-hexane partition of hydro-ethanol extracts of barks and leaves of *B. toxisperma* (Pierre) were globally the most effective extracts on tested strains. According to Adejumo and Bamidele (2009) a mycelial growth inhibition high than 60% is considered like effective in mycelial growth inhibition. The comparison of percentage of mycelial growth inhibition (Figures 1 and 2) show that at 2.5 mg/ML, the mycelial growth inhibition began to be effective on *T. soudanense* with hexane phase of water-hexane partition of hydro-ethanol extract of barks (60.82%), and with almond oil (65.65%) and Hbb (64.66%) on *T. rubrum*.

Total inhibition (100%) was observed at 5.0 mg/ml on *T. rubrum* with extracts Hbb and almond oil, and at 10 mg/ml on *T. soudanense* with extract Hbb. At different concentrations, almond oil and hexane phases of water-hexane partition of hydro-ethanol extracts of barks, and leaves of *B. toxisperma* (Pierre) extracts exhibited similar anti-trichophyton activities compared to those obtained with fluconazole which was used like reference antifungal drug (P < 0.05). The antifungal activity of almond oil would be due to the residual lipids. Many greasy substances were examined for their antimicrobial properties; it is the case of the oleic acid and the linoleic acid which have been reported to have antibacterial and antifungal activities (Skalicka et al., 2010). In their study, Chifu et al. (2010) have mentioned the fatty acids of the ω-6, ω-7 and ω-9 families and their esters to have a strong antimicrobial activity. The antifungal activity of hexane phases could be attributed to the non polar molecules which have migrated in hexane during the water-hexane partition. Hexane is known to be a non polar extracting solvent which extract non polar molecules.

At 10 mg/ml, 100% of mycelia growth inhibition was obtained with extracts of Hbb, and on *T. rubrum* with extracts of Ao and Hbb on *T. soudanense*. At 20 mg/ml, 100% of mycelia growth inhibition was also obtained with extracts Ao, Bb, Hbb, Bc, Bd, Lb and HLb on *T. soudanense*, and with extracts Ao, Bb, Hbb, Bd, HLb on *T. rubrum*. The same results were also observed at 40 mg/ml with extracts Ao, Ba, BBb, Bc, Bd, Lb, WLb, HLb and Bc on *T. soudanense*, and with extracts Ao, Bb, Hbb, Bc, Bd, Lb, HLb on *T. rubrum*.

Globally, the majority of *B. toxisperma* (Pierre) extracts exhibited their capacity to inhibit the radial growth of *T. soudanense* and *T. rubrum* on agar medium. This antifungal activity has been attributed to their chemical composition. The results obtained from phytochemical screening (Table 4) reveal that *B. toxisperma* (Pierre) contains several bioactive metabolites groups among which phenols, flavonoids, saponins, coumarin, tanins, anthocyanins, lipids and triterpenes. In studies done in recent years,
Table 5. Diameters (mm) of mycelia growth inhibition of *T. soudanense* on agar medium by *B. toxisperma* (Pierre) extracts and fluconazole after 7 days of incubation.

<table>
<thead>
<tr>
<th>Concentrations of extracts in gel (mg/ml)</th>
<th>B. toxisperma (Pierre) extracts</th>
<th>Almond oil</th>
<th>Barks</th>
<th>Leaves</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Ao</td>
<td>Ba</td>
<td>Bb</td>
<td>WBb</td>
</tr>
<tr>
<td>40</td>
<td>0.00±0.00</td>
<td>7.20±0.31</td>
<td>9.15±0.70</td>
<td>7.70±0.42</td>
<td>7.20±0.30</td>
</tr>
<tr>
<td>20</td>
<td>0.00±0.00</td>
<td>8.77±1.52</td>
<td>10.7±1.03</td>
<td>12.1±0.62</td>
<td>12.7±0.67</td>
</tr>
<tr>
<td>10</td>
<td>11.7±1.60</td>
<td>12.7±1.70</td>
<td>12.7±1.70</td>
<td>14.1±0.67</td>
<td>13.7±1.07</td>
</tr>
<tr>
<td>5.0</td>
<td>13.6±0.86</td>
<td>13.6±1.13</td>
<td>13.6±1.50</td>
<td>15.6±0.67</td>
<td>16.5±1.60</td>
</tr>
<tr>
<td>2.5</td>
<td>16.6±0.75</td>
<td>15.7±0.82</td>
<td>19.3±2.28</td>
<td>21.5±0.89</td>
<td>19.6±0.45</td>
</tr>
</tbody>
</table>

Ao = Almond oil extract, Ba = aqueous extracts, Bb = hydro-ethanol, Bc and Lc = ethanol extracts, Bd and Ld = methanol extract, Be and Le = ethyl acetate extracts, WBb and WLb = water phases of water-hexane partition, HBb and HLb = hexane phase of water-hexane partition, B = barks, L = leaves, Fluc = fluconazole. Diameter of mycelial growth of negative control = 28.5 ± 0.12 mm. Data are means of three replicates. Compared to those obtained from Fluconazole at the same concentration, diameters (mm) of mycelia growth inhibition obtained from *Baillonella toxisperma* (Pierre) extracts followed by NS, are not significantly different by Dunnett's Multiple Comparison Test. Those followed by * are significantly different at P < 0.05.

Table 6. Diameters (mm) of mycelia growth inhibition of *T. rubrum* on agar medium by *B. toxisperma* (Pierre) extracts and fluconazole after 7 days of incubation.

<table>
<thead>
<tr>
<th>Concentrations of extracts in gel (mg/ml)</th>
<th>B. toxisperma (Pierre) extracts</th>
<th>Almond oil</th>
<th>Barks</th>
<th>Leaves</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Ao</td>
<td>Ba</td>
<td>Bb</td>
<td>WBb</td>
</tr>
<tr>
<td>40</td>
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<td>7.20±0.31</td>
<td>9.15±0.70</td>
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Ao = Almond oil extract, Ba = aqueous extracts, Bb = hydro-ethanol, Bc and Lc = ethanol extracts, Bd and Ld = methanol extract, Be and Le = ethyl acetate extracts, WBb and WLb = water phases of water-hexane partition, HBb and HLb = hexane phase of water-hexane partition, B = barks, L = leaves, Fluc = fluconazole. Diameter of mycelial growth of negative control = 25.1 ± 0.39 mm. Data are means of three replicates. Compared to those obtained from Fluconazole at the same concentration, diameters (mm) of mycelia growth inhibition obtained from *Baillonella toxisperma* (Pierre) extracts followed by NS, are not significantly different by Dunnett's Multiple Comparison Test. Those followed by * are significantly different at P < 0.05.

Several authors have indicated these metabolites to be effective against fungal growth. Phenolic compound like 2-acetylfluoro-1,4-naphthoquinone, angusticomin BandbartericinA, isolated from medicinal plants, have been reported for their fungitoxic activity (Kuete, 2010). Saponins like 6α-[β-D-xylopyranosyl-(1→3)-β-Dquinovopyranosyl]-25(S)-5α-spirostan-3β-ol, isolated from the leaves of *Solanum hispidum* Pers. (Solanaceae), have been reported for their fungitoxic activity against *T. mentagrophytes* and *T. rubrum* (Gonzalez et al., 2004; Abad et al., 2007). 6-cinnamylxoyo-1-hydroxyeudesm-4-en-3-one, a sesquiterpene isolated from the roots of *Vernonia tweedieana* (Baker) H. Rob. (Asteraceae) by Portillo et al. (2005), has showed a significant antifungal against *T. mentagrophytes* (Abad et al., 2007). Flavonoids have been reported for their ability to complex with the polypeptides of microbial cell wall (Boris, 1996). Terpenoids have the property of precipitating fungal proteins (Sher, 2009; Kansole, 2009). Theses bioactive metabolites groups in *B. toxisperma* (Pierre) extracts are also known for their various physiological effects like anti-Candida, anti-bacterial, anti-oxidant, anti-inflammatory and anti-parasitic (Riwom et al., 2015; Kuete,2010; Gonzalez, 2004). The prevalence of fungal infections due to *Trichophyton* species has become very alarming these last years. Among these fungal species, *T. soudanense* and *T. rubrum* are cited in many investigations to be most virulent. These infections are typically treated by topical or systemic administration of polyene or azole antibiotics, which are efficacious but problematic.
Figure 1. Percentage of mycelial growth inhibition of *Trichophyton soudanense*. Caption: Ao = Almond oil extract, B and L = aqueous extracts, Bb and Lb = hydro-ethanol, Bc and Lc = ethanol extracts, Bd and Ld = methanol extract, Be and Le = ethyl acetate extracts, WBb and WLb = water phases of water-hexane partition, HBb and HLb = hexane phase of water-hexane partition, B = barks, L = leaves, Fluc = fluconazole.

Figure 2. Percentage of mycelial growth inhibition of *T. rubrum*. Ao = Almond oil extract, Ba and La = aqueous extracts, Bb and Lb = hydro-ethanol, Bc and Lc = ethanol extracts, Bd and Ld = methanol extract, Be and Le = ethyl acetate extracts, WBb and WLb = water phases of water-hexane partition, HBb and HLb = hexane phase of water-hexane partition, B = barks, L = leaves, Fluc = fluconazole.

(McMichael, 2001), due to their inefficacity against some trichophytic infectious and the toxicity of some of them. There is an urgency to search for new actives principles. These results obtained from this study provide a scientific validation for the ethno-medicinal uses of *B. toxisperma* (Pierre) extracts to treat fungal infections and prove that *B. toxisperma* (Pierre) contain active principles against *Trichophyton* sp. which could be isolate, and serve to develop the new antifungal therapeutic drugs.
Conclusion

During the last few years, development of drug resistance to antifungal agents in human dermatophytic fungi has increased. According to inefficacy and toxicity problems that present several current antifungal drugs, there is an urgency to search and discover new actives principles. In this study, the B. toxisperma (Pierre) extracts have showed promising antifungal activity against T. soudanense and T. rubrum. Phytochemical analysis has released that this plant contain biologically active metabolic groups such as; phenols, flavonoids, saponins, coumarin, tanins, anthocyanins, lipids and triterpenes. These metabolic groups have been reported in several studies to be used for therapeutic purposes or to serve as precursors for the synthesis of many antifungal drugs.

Conflict of Interest

The authors have not declared any conflict of interest.

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REFERENCES


Journal of Medicinal Plant Research

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