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Evaluation of phytochemical properties and *in-vitro* antibacterial activity of the aqueous extracts of leaf, seed and root of *Abrus precatorius* Linn. against *Salmonella* and *Shigella*

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ABSTRACT

Objective: To investigate the phytochemical components of *Abrus precatorius* (*A. precatorius*) and the *in-vitro* susceptibility of *Salmonella typhi* and *Shigella dysenteriae* to the aqueous extracts of *A. precatorius* leaf, seed and root.

Methods: The leaf, seed and root of *A. precatorius* were collected and homogenized separately after drying at 40 °C for seven days in hot-air oven. The aqueous extracts of each of the parts were prepared and subjected to phytochemical screening. Dilutions of 400, 300, 200, 100 mg/mL, of each of the extracts were used for broth dilution in minimum inhibitory concentration (MIC) determination against clinical isolates of *Salmonella typhi* and *Shigella dysenteriae*, while 50, 40, 30, 20, and 10 mg/mL dilutions were used for the agar diffusion test and 100 μg/mL and 10 μg/mL of gentamycin were used as controls for broth dilution in MIC determination and agar diffusion test, respectively.

Results: Qualitative study reveals that tannin, saponins, alkaloids, flavonoids, terpenoids, steroids and phenols were present in all of the plant parts. The leaf has the highest quantities of tannin and phenol. The root generally showed the lowest quantity of all the compounds. The pathogens were susceptible to aqueous extracts of the leaf, stem and root of *A. precatorius* at 50 mg/mL. At concentrations of 40, 30 and 20 mg/mL, all the aqueous extracts of *A. precatorius* showed variation in MIC, but produced no minimum bactericide effect upon subculture. There were variations in diameter of zone of inhibition against the organisms at lower concentrations.

Conclusions: These findings suggest that *A. precatorius* is a valuable source of phytochemicals with promising antibacterial activity. Considering this bioactivity, *A. precatorius* could be probed further for toxicity, and to obtain some novel antibacterial molecules.

1. Introduction

Plants are important sources of chemical compounds with potential therapeutic effects. Medicinal plants have been identified and used throughout human history for combating infectious diseases [1]. They have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions, and for defense against pests, pathogens, fungi and herbivorous mammals. At least 12 000 of such compounds have been isolated; a number estimated to be less than 10% of the total [2,3]. In 2001, researchers identified 122 compounds used in modern medicine which were derived from plants sources, and 80% of these have ethnomedicinal uses [4].

Abrus precatorius (*A. precatorius*) belongs to the class Magnoliophyta; order Fabales; family Fabaceae; subfamily Faboidene; tribe Abreae; genus *Abrus*; and of *precatorius*

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species. This plant is known with various names such as Abrus seed, Jequirity, Imisimisi, Aivoeiro, Crab eye, Rotary pea, and Indian bead among others in Africa, India, China and Brazil.

A. precatorius subspecies *Africanus* is a slender, perennial climber that twines around trees, shrubs and hedges. The leaves are glabrous with long internodes and are alternate, compound paripinnate with stipules. Each leaf is about 100–150 mm long with 20 or more leaflets. Each leaflet is about 15–25 mm long, 6–8 mm wide, oblong and obtuse. Hot or cold water extracts and dried powder of the root, leaf and stem of this plant are used traditionally as medicinal herbs. Previous reports indicated that *A. precatorius* leaf, stem and root have human and veterinary uses as antimicrobial (including *Mycobacteria tuberculosis*), antiprotozoal, insecticidal and anti-snake venom remedies [4,5]. Several groups of secondary metabolites such as alkaloids, triterpenoids, isofluranoquinones, anthocyanins, starch, tannin, flavonoids, orientin have been isolated from this plant [6,7]. These compounds may be responsible for various potential medicinal properties attributed to the plant.

Acute gastroenteritis is one of the leading causes of illness and death in infants, children and aged individuals throughout the world, especially in developing countries. Asia, Africa and Latin America had an estimated 2.5 million death annually in children less than five years of age due to infectious agents [8]. Among the enteric pathogens, *Salmonella* and *Shigella* species are of particular concern as they are responsible for enteric fever, food poisoning and gastroenteritis.

Local traditional herbal specialists use aqueous infusion or extracts (cold or hot) of leaf, seed and root of *A. precatorius* for the treatment of intestinal illnesses that could be of bacterial, viral or protozoan origins. Very few research studies have been done on the practical application of *A. precatorius* parts on clinical isolates from the intestine [9,10], these previous investigations were on different bacteria species and from other clinical sites [11]. Previous studies also used only a part of the plant [12,13]. This study therefore investigates antimicrobial activity of aqueous extracts of leaf, seed and root of *A. precatorius* on clinical isolates of *Salmonella* and *Shigella* species.

2. Materials and methods

2.1. Collection and maintenance of plant sample

The leaf, seed and root of *A. precatorius* were collected from farms in Ilorin and Igosun areas of Kwara State Nigeria. The plant was identified and authenticated by a plant taxonomist of the herbarium unit, Department of Botany, Obafemi Awolowo University, Ile-Ife, Nigeria. The parts were carefully removed, separated and dried at 40 °C for 7 days in hot-air oven (Unicorn, England). Each part was grounded to powdery form with countertop electric blender (Binatone, Japan), and stored in airtight bottles at 4 °C until it is required for use.

2.2. Preparation of extracts

The aqueous extracts of leaf, seed and root of *A. precatorius* were prepared separately as described by previous authors [14]. Two hundred grams each of the powder was suspended in 1 000 mL of sterile distilled water (SDW) in a flask. Appropriately, labeled flask was used for each plant part and placed in an orbital shaker (Gem Instrument, Japan) and agitated continuously for 8 h at 25 °C. It was then sieved with filter of pore size 30 μm. The filtrates were concentrated using rotatory evaporator (Quickfit, UK) at 80 °C, and then further evaporated in small beakers at 80 °C in water bath (Unicorn instruments, UK). The extracts were collected in airtight plastic universal bottles, labeled accordingly and stored at 4 °C till further use.

2.3. Phytochemical screening of leaf, seed and root of *A. precatorius*

Aqueous extract of *A. precatorius* leaf, seed and root were subjected to phytochemical analysis using standard techniques previously established [15]. The detection of steroids, saponins, phenolics, tannins, flavonoids, terpenoids and alkaloids were carried out respectively as previously described [16–19]. Each test was qualitatively expressed as negative (–) not present or positive (+) present; the intensity of the characteristic color was expressed as (++) or (+++) or (++++). Quantitate of steroids, saponins, phenolics, tannins, flavonoids, terpenoids and alkaloids were also determined using previously established methods [19–21].

2.4. Dilution of leaf, seed and root extracts and gentamycin

Twenty five grams of each of the extract concentrates was weighed and dissolved in 50 mL of SDW to make a dilution of 500 mg/mL as stock solution. From the stock solution, dilutions of 400, 300, 200, 100 mg/mL were made. These were used for broth dilution in minimum inhibitory concentration determination of the leaf, seed and root extracts.

From the stock, dilutions of 50, 40, 30, 20, and 10 mg/mL were prepared for each of the extracts for agar diffusion test. Gentamycin injection (Mayer and Baker, Nigeria) ampules containing 40 mg/mL was diluted serially to 100 μg/mL and 10 μg/mL and these were used as control drug for broth dilution and agar diffusion tests, respectively.

2.5. Test organisms

Clinical isolates of *Salmonella typhi* (*S. typhi*) and *Shigella dysenteriae* (*Sh. dysenteriae*) were obtained from the Department of Microbiology and Parasitiology, University of Ilorin Teaching Hospital, Ilorin, Kwara State, Nigeria. The organisms were isolated and confirmed from stool samples of patients with intestinal illnesses. The isolates were maintained on nutrient agar plates at 4 °C and were sub-cultured onto nutrient agar for 24 h before *in-vitro* microbial test commenced. Standard inoculum was prepared in sterile normal saline to 0.5 Mcfarland standard of 1×10^6 CFU/mL.

2.6. Dilution of nutrient broth

For each of the extract, 9 mL of nutrient broth were prepared in separate McCartney bottles from the serial dilution of 500, 400, 300, 200, 100 mg/mL previously prepared and 100 μg/mL of gentamycin and SDW were used as controls. One milliliter of each of these was added to 9 mL of nutrient broth in the separate bottles to give a final concentration of 50, 40, 30, 20, 10 mg/mL of each extract. One milliliter of each of 100 μg/mL of gentamycin and SDW were used as control. Each test organism was prepared by adding a drop of standardized organism suspension to all bottles prepared using sterile Pasteur pipette. All bottles

were incubated at 37 °C for 18–24 h. Minimum inhibitory concentration was determined by checking for the lowest dilution in which there was no turbidity [22].

All bottles with no turbidity were sub-cultured on Mueller Hinton agar and incubated at 37 °C for 18–24 h to determine minimum bactericidal concentration. The lowest dilution that showed no growth on agar plate was taken as minimum bactericidal concentration [22].

2.7. Agar well diffusion

Three plates, each for leaf, seed and root of the extracts, were used for the agar diffusion test for each of the test organisms. Mueller Hinton agar plates previously prepared and allowed to dry in the incubator at 37 °C for 30 min were used. The plates were seeded with sterile swab stick dipped in diluted culture to 1×10^6 CFU/mL of each of the test organisms and spread on the entire surface of the plate to give semi-confluent growth. Using a sterilized cork borer, six holes of 5-mm diameter were bored in circle around a central hole on the seeded agar plate at equal distance apart. Six drops of each dilution was dispensed into appropriately labeled hole while the center hole received six drops of 10 μg/mL dilution of gentamycin. The same procedure was repeated for all the extracts in triplicates for each of the organism. All the plates were left on the surface of the inoculating chamber for 2 h to allow diffusion of extracts and antibiotics. The plates were then incubated at 37 °C aerobically for 18–20 h. The zone sizes were measured to the nearest millimeter and averaged. It was interpreted as described by previous studies [23,24].

3. Results

Seven phytochemicals, namely, tannin, saponins, alkaloids, flavonoids, terpenoids, steroids and phenols were assayed in aqueous extracts of leaf, seed and root of *A. precatorius*. The phytochemicals were detected in all the three parts. The three parts were very rich in tannin and phenols but low in alkanoids, flavonoids and steroids (Table 1).

Quantitative analyses of the constituents of these phytochemicals showed that the leaf had the highest quantity of tannin and phenol with 27.71 mg/g and 22.84 mg/g, respectively. The root generally showed lower quantities of the seven phytochemical assayed than the leaf and seed as shown in Table 2.

There were differences in susceptibility of *S. typhi* and *Sh. dysenteriae* to various concentrations of aqueous extracts of *A. precatorius* leaf, seed and root. Generally, the susceptibility of both organisms was concentration dependent. The higher the concentrations used, the higher the susceptibility of the organisms. *S. typhi* is more sensitive to the three parts of the plants than *Sh. dysenteriae*. The two intestinal pathogens were sensitive to gentamycin at 10 μg/mL than the highest concentration of the aqueous extracts of the three parts of *A. precatorius* as depicted in Table 3.

The zone diameter of inhibition of each extract concentration was measured in nearest mL [24], and this was compared with the control drug (gentamycin 10 μg/mL). *S. typhi* is sensitive to aqueous extracts of leaf, seed and root at higher concentration of dilution (50 mg/mL) as shown in Table 3. The extracts were resistant to lower concentrations of the extracts of leaf,

Table 1
Qualitative constituents of aqueous extract of leaf, seed and root of *A. precatorius*.

Plant parts	Tannin	Saponins	Alkaloids	Flavonoids	Terpenoids	Steroids	Phenols
Leaf	+++	++	+	+	++	+	+++
Seed	+++	+++	+	+	+	+	+++
Root	+++	++	+	+	+++	+	++

Grades of color development: +: Light; ++: Moderate; +++: Deep.

Table 2
Quantity of phytochemicals in aqueous extracts of leaf, seed and root of *A. precatorius* (mg/g).

Plant parts	Tannin	Saponins	Alkaloids	Flavonoids	Terpenoids	Steroids	Phenols
Leaf	27.71	11.00	6.18	3.73	10.23	4.27	22.84
Seed	21.34	17.80	4.06	3.68	6.73	5.17	17.08
Root	12.80	4.55	2.18	1.41	3.63	2.08	10.87

Table 3
Antibacterial activity of aqueous extract of leaf, seed and root of *A. precatorius* (mm).

Extract concentration (mg/mL)	Zone diameter of inhibition					
	Leaf		Seed		Root	
	S. typhi	*Sh. dysenteriae*	*S. typhi*	*Sh. dysenteriae*	*S. typhi*	*Sh. dysenteriae*
50	17	15	19	17	16	15
40	13	9	16	14	12	10
30	9	6	13	11	10	6
20	5	5	7	5	9	5
10	5	5	5	5	9	5
SDW	5	5	5	5	5	5
Gentamycin (10 μg/mL)	24	22	23	21	25	23

Table 4
Minimum inhibitory concentration of aqueous extract of leaf, seed and root of *A. precatorius*.

Extract concentration (mg/mL)	Leaf		Seed		Root	
	S. typhi	*Sh. dysenteriae*	*S. typhi*	*Sh. dysenteriae*	*S. typhi*	*Sh. dysenteriae*
50	NG	NG	NG	NG	NG	NG
40	NG	NG	NG	NG	G	G
30	G	G	NG	G	G	G
20	G	G	G	G	G	G
10	G	G	G	G	G	G
SDW	G	G	G	G	G	G
Gentamycin	NG	NG	NG	NG	NG	NG

G: Growth observed; NG: No growth observed.

seed and root. Extracts of seed have higher inhibitory effects than the leaf and root on *S. typhi*.

Sh. dysenteriae was sensitive only to aqueous extract of seed, but reduced susceptibility to aqueous extracts of leaf and root at 50 mg/mL. It was, however, resistant to other concentrations tested as evidenced in Table 3. At concentrations of 50 and 40 mg/mL, the three parts of *A. precatorius* extracts produced inhibition in broth culture of *S. typhi* and *Sh. dysenteriae* (Table 4). The aqueous extract of the seed produced inhibition at 30 mg/mL as exhibited in Table 4. Both organisms grew at concentrations that produced minimum inhibitory concentrations in the minimum bactericidal concentration test.

4. Discussion

Many useful drugs have been formulated through the exploration of whole or parts of medicinal plants. This exploration will definitely continue as long as microorganisms keep developing drug resistance. Previous works showed that *A. precatorius* is a unique source of many potential phytochemicals that can be explored for human use. In this study, we have found seven phytochemicals in the three parts of *A. precatorius*. These include alkaloids, flavonoids, phenols, saponins, steroids, tannins and terpenoids. There are slight variations in the concentrations of these phytochemicals in the plant parts. The leaf and seed of the plant have higher concentration of phytochemical than the root (Table 2).

The plant has high quantities of tannin and phenols, but lower concentration of other phytochemicals as indicated in Tables 1 and 2. Similar reports were obtained by other authors [5,6]. Several other compounds such as abine, anthocyanins, abrectori, and isorienlin have been found in *A. precatorius* [25]. These compounds might have contributed to its various medicinal uses such as antimicrobial, antiprotozoan and antihelminthic [6].

Antibacterial activity of *A. precatorius* against *S. typhi* and *Sh. dysenteriae* showed that the aqueous extract of the plant parts have antibacterial activity against them (Table 3). The antibacterial activity of seed of *A. precatorius* has more profound effect than the leaf and root. At concentration of 50 mg/mL, the inhibitory activity of aqueous extracts of seed of this plant against *S. typhi* and *Sh. dysenteriae* is similar to that of the control. The extract had zone of inhibition of diameter 17 mm and 19 mm against *S. typhi* and *Sh. dysenteriae* while the control has zone inhibition diameter of 23 and 21 against the respective organism [24]. At concentration of 40 mg/mL the aqueous extracts produce varied zone of inhibition (Table 3). These were however, lower than zone diameter sizes produced by the control the organisms. This result is comparable to the findings of previous authors [10], who reported moderate antimicrobial activity of seed of *A. precatorius* against clinically important bacteria such as *Enterococcus faecalis*, *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus thermophilus*, *Pseudomonas aeruginosa* and *Micrococcus luteus*.

The aqueous extract of the seed of *A. precatorius* has slightly higher concentration of phytochemicals than the leaf and root, which may contribute to it higher antimicrobial activity. Earlier study revealed that the seed of *A. precatorius* has diverse phytochemical components [26]. The phytochemical components of aqueous extracts of leaf were also slightly higher than those of root (Tables 1 and 2). This is also reflected in the antibacterial susceptibility of the tested organisms, and this could justify the reason why local herbal practitioners prefer to use the leaf and seed of the plant for various ailment treatments.

At concentrations of 50, 40 and 30 mg/mL, the aqueous extracts that produced inhibition of growth of these organisms in nutrient broth failed minimum bactericidal concentration determination test. This indicated that the aqueous extracts of the parts of *A. precatorius* only inhibit but does not kill the organisms at the concentrations used.

However, it has been reported that the seed of *A. precatorius* can be extremely poisonous, especially if cracked before consumption. High toxic level can cause severe stomach cramps, diarrhea, tachycardia, coma, cold sweat and nausea [9,27]. Generally, aqueous extracts or infusion of any part of *A. precatorius* must be taken with caution because prolong use of this plant has been associated with anemia and can increase the level of human white blood cell count [28–30]. Therefore, it is strongly advised that aqueous extracts or any product from *A. precatorius* should be taken under professional guidance.

Further research work is needed on phytochemical components present in this plant that may be injurious to human health; and there is a need to determine the bioactive components that are of medicinal value which can be extracted for human use.

In conclusion, this study has offered a scientific basis for traditional use of aqueous extracts of *A. precatorius* for treatment of gastrointestinal bacterial infections. Further study is necessary to determine toxicity level of the bioactive component and possibly remove the toxins and determine active phytochemical components for drug production.

Conflict of interest statement

We declare that we have no conflict of interest.

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