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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