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Original article

THE PHYTOCHEMICAL CONSTITUENTS AND ANTIMICROBIAL ACTIVITIES OF ABRUS PRECATORIUS METHANOL LEAF AND SEED EXTRACTS

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ABSTRACT

Abrus precatorius commonly known as rosary pea in English and *Idon Zakara* in Hausa; Nigeria, has been used in traditional medicine. The study was carried out to determine the phytochemical constituents and the *in vitro* antimicrobial activities of *Abrus precatorius* (*A. precatorius*) methanol seed and leaf extracts. The quantitative phytochemical contents were determined using standard methods. Different concentrations of the extracts (120mg/ml, 160mg/ml and 200mg/ml) were prepared to determine their antimicrobial activity against *Staphylococcus aureus, Salmonella typhi, Klebsiella pneumonia, Penicillum spp., Aspergillus niger* and *Trychophytom spp.* A double fold serial dilution (200 – 12.5 mg/ml) was used to determine the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the extract. The quantitative phytochemical contents of methanol seed extract of *A. precatorius* showed that phenols had the highest concentration (563.95±0.15 mg/100g) and alkaloid had the least concentration (34.15±0.38 mg/100g), while in methanol leaf extract, Saponins had the highest concentration (2560.64±11.77 mg/100g) and Tannins had the least concentration (35.05±0.14 mg/100g). The bacterial isolates were all susceptible to methanol seed extracts of *A. precatorius* at a concentration of 200mg/ml, showing the highest zone of inhibition for *Klebsiella* (26mm) and *S. typhi* (21mm) exhibiting the least zone of inhibition for the leaf extract, while all the fungi isolates were resistant to the extracts at the concentration tested. The result for MIC methanol seed extract showed highest MIC value for *Klebsiella* as 200mg/ml and the least 175mg/ml for both *S. typhi* and *S. aureus* while 200mg/ml MIC value for *Klebsiella*, *S. typhi* and *S. aureus* in methanol leaf extract. There was no significant difference (at $p>0.05$) between MIC and MBC of *K. pneumonia* while significant difference was observed in the treatment with methanol seed and leaf extract for both *S. typhi* and *S. aureus.* Based on the result obtained from this study, it can be concluded that the methanol seed and leaf extracts of *A. precatorius* have inhibitory potential against bacterial isolates. These observed level of activity may be

attributed to presence of phytoconstituents in extracts of *A. precatorius*. Therefore, *A. precatorius* could serve as antibacterial agents.

Keywords: *Abrus precatorius,* Bacterial & Fungal pathogens, Minimum Bactericidal Concentration (MBC), Minimum Inhibitory Concentration (MIC), Phytochemical Contents

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INTRODUCTION

Infectious diseases caused by pathogenic microorganisms are currently the world's leading causes of premature deaths, killing almost 50,000 people every day [1]. These pathogens have the ability to infect human and animals, thereby reducing the quantity or product turn out of agricultural produce by destroying crop plants [2, 3]. Bacterial disease causes loss in the quality and quantity of vegetable crops every year. The bacteria *Pectobacterium carotovorum subsp carotovorum (Pcc) (syn. Erwinia carotovora subsp. carotovora)* is the cause of soft rot disease in potato, [4].

The control of these diseases has posed new challenges because of the emergence of multidrug resistance among several pathogens to some of the antimicrobial drugs due to misuse [5]. In addition to this problem, some antibiotics are sometimes associated with adverse effects on the host including hypersensitivity, immune-suppression, allergic reactions and even loss of hearing [1, 6]. This situation necessitates the continued search for new antimicrobial substances. Much attention is now concentrated on plant extracts with biologically active compounds isolated from plant species as reported by [7]. Antimicrobials of plant origin have enormous potentials. They are noted to be effective in the treatment of infectious diseases particularly bacterial infection, which is one of the most serious global health issues arising in the 21st century. In addition to the effective treatment of infectious diseases, medicinal plants simultaneously mitigate many of the side effects that are often associated with synthetic antimicrobials [8].

Herbal medicines are in great demand for primary healthcare because of their wide biological and medicinal activities, higher safety margins and lesser costs. Several plants used in traditional medicine have been studied for antimicrobial activity to develop a source of new antifungal and antibacterial compounds with fewer side effects, a wider spectrum of action and lower cost [9, 10, 11].

Abrus precatorius is a medicinal plant belonging to the family *Fabaceae.* It is known as rosary pea in English but has several common names. The plant grows in tropical climates such as India, Sri Lanka, Thailand, the Philippine Islands, South China, tropical Africa but it is also found in most West African countries including Nigeria. It is one of the important herbs reported to have a broad range of therapeutic effects, like anti-bacterial, anti-fungal, anti- diabetic, anti-migraine, including treatment of inflammation, ulcers, wounds, throat scratches, sores to mention a few [12]. It is also considered as a valuable source of unique natural products (phytochemicals) for development of medicines against various diseases and also for the development of industrial products. These phytochemicals have been suggested to be responsible for

the medicinal properties observed in most medicinal plants [13].

MATERIALS AND METHODS

Plant collection and identification

Dried seed and leaf of *Abrus precatorius* plant were obtained from Kuta and Kuchiworo, Niger State, Nigeria between the month of July and August 2019.The plant *Abrus precatorius* was deposited at the herbarium of University of Ilorin Kwara state, Nigeria for identification and voucher number was allocated as UILH/001/2019/574.

Sample preparation and extraction

The dried seed and leaf were milled into powder–form with an electrical grinder and stored in an airtight container. Five hundred gram (500g) each of the powdered sample was macerated with 3500ml of methanol at room for 72hours modified method of [14]. The extract was filtered (Whattman no1), concentrated and stored in sample bottles in the refrigerator at 4^{o}C until required [15].

Quantitative phytochemical analysis

Quantitative phytochemical analysis of methanol extract of *Abrus precatorius* plant was carried out using spectrophotometer (Shimadzu UV-S1800) to determine the amount each of phenols, alkaloids, tannins, saponins and flavonoids present, as described by [16, 17, 18].

Preparation of media

All the media used were prepared according to the manufacturer's instructions. Briefly, accurately weighed 18.0g of nutrient agar NA (Accumedia) and 37g of saboroud dextrose agar SDA (TM Media) were dissolved in 500ml of distilled water respectively. Two chloramphenicol capsule (500 mg) were added into the SDA and shook. The media were sterilized at 121^{o}C for 45mins in an autoclave. The autoclave was allowed to stand for about 1 hour in order for the pressure to come down. The media were brought out of the autoclave and allow to cool to a holding temperature. On cooling, 30ml of the sterilized media were dispensed into into 90mm petri dishes under aseptic conditions in laminar flow. The plates were allowed to cool at room temperature to solidify the media.

Preparation of inocula

Three test tubes containing 5ml each of nutrient broth and three tubes containing 10ml of Sabouraud Dextrose Broth (SDB) medium were sterilized in the autoclaved. Active cultures for the experiments were prepared by transferring a loopful of bacterial culture into 5ml of nutrient broth and loopful of fungal spore into 10ml of SDB medium and shook to allow for the organisms' proliferation. The inoculum was incubated at 37^{o}C for 24hours for bacteria and 25^{o}C for 24hours for fungi.

Dilution of seed and leaf extracts

Exactly 0.6g, 0.8g, and one gram (1.0g) of the seed and leaf extracts were weighed and dissolved in 5ml of dimethylsulfoxide (DMSO) each to obtain a concentration of 120mg/ml, 160mg/ml and 200mg/ml respectively.

Antimicrobial Sensitivity Test

Agar well diffusion method was employed for both bacteria and fungi. Appropriate standardized culture of the test organisms were seeded onto nutrient agar and SDA

(media) plates and uniformly spread with a sterile swab stick dipped into the culture of each of the test organisms. Three wells of 5mm diameter were bored onto the inoculated plates using sterile cork borer. They appropriately labeled wells were filled with the extract. All the plates were left on the surface of the inoculating chamber at room temperature for 1 hour allowing for diffusion of the extract. Diameter of zone of inhibition on agar surface were determined after incubating plates at 37^{o}C for 24hours (bacteria) and 25^{o}C for 72hr (fungus) (Rice and Bonomo, 2007).

Minimum inhibitory concentration (MIC)

The MIC was determined using the nutrient broth dilution technique as described by [19]. Two-fold serial dilution of the extract was then made to a concentrations ranging from 12.5mg/ml, 25mg/ml, 50mg/ml, 100mg/ml and 200mg/ml. The extract was first diluted to the highest concentration (200mg/ml) using DMSO. 2ml of the extract concentration was added into nutrient broth and then 2.0 ml of standardized broth cultures containing a loopful of the organisms were seeded into each test tube and then incubated at 37^{o} C for 18-24 hours. The minimum inhibitory concentration value was determined for the microorganisms that were sensitive to the extract under study. MIC was defined as the lowest concentration where no turbidity was observed in the test tubes.

Minimum Bactericidal Concentration (MBC)

The MBC was determined using the broth dilution technique previously described by [20] by assaying the test tubes resulting from MIC determinations. A 1oopful of the content of each test tube was then inoculated by streaking on a solidified nutrient agar plate and then incubated at 37 ^{o}C for 24 hours for possible bacterial growth. The lowest concentration of the sub-culture that shows no bacterial growth was considered the minimum bactericidal concentration of the plant against the test organisms.

RESULTS

The result of quantitative phytochemical revealed higher content ($P < 0.05$) concentrations of flavonoids, phenols, tannin, saponins, in *Abrus precatorius* methanol seed extract than *Abrus precatorius* methanol leaf extract. Significantly higher ($P < 0.05$) alkaloids content was observed in leaf than seed as shown in table 1.

Table 1: Secondary metabolites compositions of *A. Precatorius* methanol seed and leaf extracts (mg/100g)

Secondary metabolites	Seed (mg/100g)	Leaf (mg/100g)
Phenols	563.95 ± 0.15^{b}	286.88 ± 0.48^{a}
Flavonoids	146.50 ± 1.44^{b}	141.40 ± 0.10^{a}
Alkaloids	34.15 ± 0.38^{a}	109.65 ± 1.13^{b}
Tannins	52.77 ± 0.74^{b}	35.05 ± 0.14^{a}
Saponins	294.05 ± 0.09^{b}	256.64 ± 11.77^{a}

Values are presented as mean $\pm$ standard deviation of three replicates.
Values with the same superscript in a row are not statistically different at $p > 0.05$.

The zone of inhibition of methanol seed and leaf extracts of *A. precatorius* against some microbial isolate are presented in table 2. The result revealed inhibition of *Salmonella typhi, Klebsiella pneumonia* and *Staphylococcus aureus* at 200 mg/ml while no inhibition of the fungi isolates were observed for both *A. precatorius* methanol seed and leaf extracts.

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) mg/ml of *A. precatorius* methanol seed and leaf extracts against some microbial isolates as revealed in table 3 occurred at 200 mg/ml for *Salmonella typhi, Klebsiella pneumonia* and *Staphylococcus aureus* treated with methanol leaf extracts of *A. precatorius*. While the MIC and MBC for methanol seed extracts of *A. precatoius* occurred at 175 mg/ml for *Salmonella typhi* and *Staphylococcus aureus* and *Klebsiella pneumonia* at 200 mg/ml.

Table 2: Zone of inhibition of methanol seed and leaf extract of *A. precatorius* against some pathogenic microorganisms.

	Zone of inhibition (mm)					
	Seed			leaf		
Concentrations (mg/ml)	120	160	200	120	160	200
Test organisms						
Salmonella typhi	-	-	21 ± 0.04^{a}	-	-	24 ± 0.14^{b}
Klebsiella pneumonia	-	-	26 ± 0.25^{b}	-	-	24 ± 0.24^{b}
Staphylococcus aureus	-	-	24 ± 0.12^{a}	-	-	26 ± 0.18^{b}
Aspergillus niger	-	-	-	-	-	-
Trychopytom spp	-	-	-	-	-	-
Penicillum spp	-	-	-	-	-	-

Values are presented as mean ± standard deviation of three replicates.
Values with the same superscript in a row are not statistically different at $p > 0.05$

Table 3: Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) mg/ml of methanol seed and leaf extracts of *A. precatoius* against some pathogenic microorganisms

Microorganisms	MIC (mg/ml)		MBC (mg/ml)	
	Seed	Leaf	Seed	Leaf
Salmonella typhi	175 ± 0.23^{a}	200 ± 0.51^{b}	175 ± 0.18^{a}	200 ± 0.22^{b}
Klebsiella pneumonia	200 ± 0.45^{a}	200 ± 0.39^{a}	200 ± 0.38^{a}	200 ± 0.29^{a}
Staphylococcus aureus	175 ± 0.27^{a}	200 ± 0.34^{b}	175 ± 0.44^{a}	200 ± 0.14^{b}

Values are presented as mean ± standard deviation of three replicates.
Values with the same superscript in a row are not statistically different at $p > 0.05$

DISCUSSION

Infectious diseases are still a major challenge to health issues all over the world. The emergence of drug resistant microorganisms has further compounded the problem [1]. Therefore, the need to use plant extracts with known antimicrobial properties to improve the efficiency of treatment in traditional medicine is of importance [5].

In this study, the quantitative phytochemical analysis of methanol seed and leaf extracts of *A. precatorius* were revealed to be; flavonoids, alkaloids, tannins, saponins and phenols, which is in accordance with earlier studies that revealed the presence of diverse phytochemical constituents in *A. precatorius* (Madaki *et al.,* 2019; Arora *et al*., 2011). However, the seed has shown significantly ($p < 0.05$) higher amounts of phenols, saponins, flavonoids and tannins than the leaf extract while alkaloids content is significantly higher ($p < 0.05$) in methanol leaf extract. This finding is in contrast with result of [21], which revealed the presence of high amount of tannins. However, the methanol leaf extract had the highest saponins content with tannins being the least phytoconstituents which is agreement with the study of [22]. All these phytochemicals observed had contributed to the *in vitro* antimicrobial activity of the plant. Tannins have been reported to inhibit growth of microorganisms by precipitating microbial protein and making nutritional protein unavailable to them [23]; while the antimicrobial effects of flavonoids have been attributed to their ability to form complex with extra cellular, soluble protein and with bacterial cell wall proteins [24]. The flavonoids have been known to be synthesized by plants in response to microbial infection [25]. Saponins also have been reported to exhibit wide range of biological activities especially antibacterial [25] whose mode of action involves cell membrane lysis.

The *in vitro* antimicrobial activity of the methanol seed and leaf extracts of *A. precatorius* against three bacterial isolates namely: *Salmonella typhi* (*S. typhi*), *Klebsiella pneumoniae, Staphylococcus aureus* showed that both the leaf and seed extracts had antibacterial activity against all the tested bacteria at a concentration of 200mg/ml, while against three fungal isolates namely *Aspergillus niger, Trychophytom,* and *Penicillum spp* showed no activities at the concentration tested. This could be due to the variation in plant part, solvent of extraction and concentrations of extract used. At this point inhibitory test conduct to ascertain the minimum inhibitory concentration between of 160mg/ml and 200mg/ml. The extracts had zone of inhibition for both the seed and leaf respectively, which shows that *Klebsiella pneumoniae* had higher MIC and MBC was less sensitive than the other organisms tested, while *Staphylococcus aureus* and *Salmonella typhi* were more sensitive to the seed extract. The antibacterial activity of the extract of *A. precatorius* might be due to the presence of phytochemicals such as flavonoids, alkaloids, tannins, saponins and phenols as proven by many authors including; [26, 27, 28].

The result of the microbial studies reveals that *A. precatorius* methanol seed extract was significantly ($p < 0.05$) more active than the leaf extract. However, minimum inhibitory concentration (MIC) and

minimum bactericidal concentrations (MBC) result showed that the *A. precatorius* methanol seed possess antibacterial activities against all the tested bacteria.

CONCLUSION

The study demonstrated that the *A. precatorius* methanol seed and leaf extracts possessed inhibitory activities against the bacterial isolates tested. These activities could be as a result of phytochemicals present in the *A. precatorius*. In addition, the study has provided a scientific evidence and confirmed the use of these plant in the treatment of infectious diseases

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