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Antibacterial activity of leaves and root extracts of *Abrus precatorius*

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Abstract
This present study was designed to evaluate the antibacterial potential and to determine the zone of growth inhibition of leaf and root extracts of *Abrus precatorius* on some bacterial strains. The antibacterial activity was evaluated against three bacterial strains, *Staphylococcus aureus* (*S. aureus*), *Escherichia coli* (*E-coli*) *and Salmonella typhii* (*S. typhii*) after successive maceration in four solvents (hexane, dichloromethane (DCM), ethyl acetate and methanol). Agar disc diffusion method was used to determine the antibacterial activity of extract (50, 100, 250 and 500 µg/ml) against human pathogenic bacteria. Zone of growth inhibition of extracts were compared with that of standard drug chloramphenicol and DMSO using a statistical software SPSS 22 and the result was expressed at 95 % level of confidence. A remarkable bactericidal activity of the leaf extract was observed in the extracts against the growth of the test organisms with mean values of zone of growth inhibition ranging from 10 mm to 12, except in methanol extract against *E-coli,* with the mean value of zone of growth inhibition is 6 mm at 500 µg/ml. Antibacterial activity was recorded for the root extract against the test organisms with mean values of zone of growth inhibition ranging from 10 mm to 12 mm, however hexane and DCM extract did not exhibit significant antibacterial activity against *E-coli.* The result of the study thus indicated the leaf and root extracts of *Abrus precatorius* contains phytochemicals that are potential antibacterial sources and can be used to discover bioactive products that may be useful in combating bacteria.

Keywords: Antibacterial activity, *Abrus precatorius,* bacterial strain, Zone of inhibition, organic solvents.

1. Introduction
For centuries, herbal medicine has been known to man and the therapeutic efficacy of many indigenous plants used in treatment of several disorders and infections has been described by various practitioners of traditional medicine [1-3]. Harmful microorganisms are being treated/controlled with synthetic drugs, and continuous treatment results in emergence of multiple drug resistance bacteria [4, 5]. This creates an alarming clinical situation in the treatment of infections [6], thereby turned researchers to plant sources to search for the active molecules that could combat these new trends in health challenges. Thus, plants hold the key to the discovery and development of new pharmaceutical and biological resources that will champion the course of health and well-being of human. Antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world by researchers [7, 8]. This aid pharmacological industries in discovery of potential sources of combating the drug resistance bacteria from natural sources [9]. Many medicinal plants used to combat different diseases possess antimicrobial activities [10-12]. *Abrus precatorius* is a medicinal plant that is used in management and treatment of many diseases, it is widely found in Africa, India and many other parts of the world. The leaf has been reported to have a characteristic sweet taste and have been employed as sweetener in food and certain medicines [13]. Published reports showed it protects the liver against CCl4 induced liver damage in rats [14], exhibit anti-HIV [15], anti-tumour, immunomodulatory [16], anti-ulcerative and anti-inflammatory properties [17-19]. The quest thereof to contribute to the novelty of this medicinal plant, and to boost the search for the discovery of potential sources of combating the drug resistance bacteria. This necessitates the study for antibacterial potential of the leaf and root extracts of *Abrus precatorius* from different solvent of different polarity.

2. Materials and Methods

2.1 Preparation of plant extract.

The freshly dried leaves and root of *Abrus precatorius* were grounded into powdered forms using laboratory grinder machine (FGR-350, Quest Scientific), serial extraction was done using four different solvent systems in order of increasing polarity (n-hexane, dichloromethane (J.T. Baker), ethyl acetate and methanol (Merck KGaA). 100 g of the powdered sample was weighed into an Erlenmeyer flask and each solvent (three times the weight of the extracts) was added successively, the solution was covered and shaken at time interval of an hour and then allow to stand for 7 days at room temperature (23 ^{o}C to 25 ^{o}C). The mixture was then filtered using Whatman filter paper No.4 and the solvent was evaporated using a rotary evaporator (Heidolph Laborato 400 Germany) under reduced pressure below 50 ^{o}C. The stock solution of the extracts (5 mg/ml) was prepared by dissolving known amount of the dry extract in 98% methanol. Working solution of each extract (1, 10, 50, 100, 500 and 1000 ppm) was prepared from the stock solution using suitable dilution.

2.2 Test microorganisms

Bacterial strains *Escherichia coli* (*E-coli*), *Salmonella typhii* (*S. typhii*), *Staphylococcus aureus* (*S. aureus*) were selected for the study. The bacterial strains were obtained from the microbiology laboratory, Faculty of Resource Science and Technology Universiti Malaysia Sarawak, and were used for the antimicrobial activities. The stock cultures of the was incubated at 37 ^{o}C for 24 hours on nutrient agar (Microcare laboratory, Surat, India), and was stored at 4 ^{o}C. Plates containing Mueller-Hinton agar (MHA) were used to grow the bacterial strains at 37 ^{o}C. The stock cultures were then kept at 4 ^{o}C.

2.3 Antibacterial activity (Determination of zone of nhibition)

Antibacterial activity of leaf and root extracts *Abrus precatorius* was determined against three pathogenic bacterial strains *E-coli*, *S. typhii* and *S. aureus* using agar disk diffusion method as reported by various authors. [4, 20] The extract was dissolved using dimethyl sulfoxide (DMSO) and sterilised by filtration and stored at 4 ^{o}C. Standard antibiotic (chloramphenicol) was used for comparison of the zone of inhibition of the pure strains of the bacteria. The extracts were then screen for their antimicrobial activity against the bacterial strains. Set of four dilutions for antibacterial activity (50, 100, 250, 500 µg/ml) of the leaf and root extracts of *Abrus precatorius* and the standard drug was prepared in distilled water. Sterile plates containing Mueller-Hinton agar were seeded with indicator bacterial strains and control experiment using chloramphenicol as standard drug were kept for 3 hours at 37 ^{o}C. They were then incubated for 18 to 24 hrs at 37 ^{o}C, and the zones of growth inhibition around the disks were measured in mm. ss

The antibacterial activity of the test organisms on the plant extracts were determined by measuring the size of the inhibitory zones (this include the diameter of the disk) on the surface of the agar around the disk, and the values <9 mm were considered as not active against the microorganism for antibacterial activity. The experiment was carried out in triplicate and the mean values of the diameter of zones of inhibition was calculated.

2.4 Statistical analysis

The mean values for the zones of growth inhibition of the plant extracts were calculated using a statistical software SPSS 22 and the result was expressed at 95 % level of confidence ($p < 0.05$).

3. Result and Discussion

The result in Table 1 below show a remarkable bactericidal activity of the leaf extract of *A. precatorius* was observed against the growth of the test organisms with mean values of zone of growth inhibition ranging from 10 mm to 11 mm against *E-coli*, 9 mm -12 mm against *S. typhii* and 10 mm to 12 mm against *S. aureu*s, as compared to the standard drug chloramphenicol at 500 µg/ml. An exception was observed as not to exhibit significant antibacterial activity in methanol fraction of the leaf extract against the bacterial strain *E-coli* at 500 µg/ml. It gives a mean value of zone of growth inhibition is 6 mm as compared to the standard drug chloramphenicol.

Table 1: Mean values of the antibacterial activity of the leaf extracts of *Abrus precatorius* at different concentration at 500 µg/ml of Chloramphenicol

Bacteria spp	Diameter of zone of inhibition in mm Concentration in µg/ml solvents					
	Chloram-phenicol	DMSO	Hexane	DCM	Ethyl acetate	Methanol
E-coli	20	2	10*	11*	11*	6
S. typhii	20	2	12*	10*	10*	9*
S. aureus	20	2	11*	12*	10*	12*

Result is Mean ± SD. N = 3, *= significant activity observed

Table 1. above show the mean values of zone of inhibition of the antibacterial activity of extracts of Abrus precatorius leaves against bacterial strains in mm. Significant activity was observed in all the extracts at 500 µg/ml except for S. aureus in methanol extract

Table 2 below show the root extract of *A. precatorius* also exhibit antibacterial activity against the test organisms with mean values of zone of growth inhibition ranging from 10 mm to 11 mm against *E-coli*, 10 mm -11 mm against *S. typhii* and 10 mm to 12 mm against *S. aureu*s as compared to the standard drug chloramphenicol at 500 µg/ml. However, the result from hexane and DCM fractions of the root extract did not exhibit significant antibacterial activity against *E-coli* at 500 µg/ml as compared to the standard drug chloramphenicol.

Table 2: Mean values of the antibacterial activity of the root extract of *Abrus precatorius* at different concentration at 500 µg/ml of Chloramphenicol.

Bacteria spp	Diameter of zone of inhibition in mm Concentration in µg/ml solvents					
	Chloram- phenicol	DMSO	Hexane	DCM	Ethyl acetate	Methanol
E-coli	20	2	7	8	11*	10*
S. typhii	20	2	11*	10*	10*	11*
S. aureus	20	2	12*	10*	10*	10*

Result is Mean ± SD. N = 3 *= significant activity observed

Table 1. above show the mean values of zone of inhibition of the antibacterial activity of extracts of Abrus precatorius leaves against bacterial strains in mm. Significant activity was observed in all the extracts at 500 µg/ml except for S. aureus in methanol extract

Related literature have shown that several authors have reported on the effectiveness of medicinal plants in different solvents against the pathogenic bacterial strains [4, 11, 20, 21-28], however the activity of the leaf extract in methanol fraction against *E-coli* was not in congruent to the finding of Ihsan-UL-Haq [23] in which they reported that the leaf extract of the methanol fraction was effective against *E-coli*, this might be because of the concentration of the extract used may be little to exhibit remarkable activity. This might also be assumed for the insignificant activity observed in the root extract in hexane and DCM fractions. Therefore, the findings in this study shows the leaf and root extracts of *A. precatorius* has the potential to be used as a valuable source of treating infections and natural source for the discovery of antibacterial agents.

4. Conclusion

The result obtained from this study revealed the leaf and root extracts of *A. precatorius* contains potential antibacterial agents, this shows the plant can be a valuable natural source for the treatment and discovery of novel phytochemicals that could be effective against antimicrobial infections and drug resistant microorganism.

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