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The Effects of Securinega Virosa Leaves on Methicillin-Resistant Staphylococcus Aureus (MRSA)

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ABSTRACT

The goal of this study was to evaluate the comparative effect of Securinega virosa plant extract and partitioned fractions on Methicillin-resistant Staphylococcus aureus (MRSA). S. virosa Leaves were extracted with 80% methanol. Phytochemical screening of the extract was performed using standard analytical methods. The antimicrobial activity of the crude extract and partitioned fractions were evaluated by the agar well diffusion method against the strain of MRSA. Also, the minimum inhibitory concentration of crude extract and partitioned fractions were evaluated.
Phytochemical screening of the Securinega virosa extract (leaf) showed the presence of alkaloids, flavonoids, saponin, steroids, tannins, terpenes, cardiac glycosides, anthraquinones, and reducing sugars. Antibacterial evaluation of crude extract of S. virosa at concentrations of 100, 50, 25, 12.5, and 6.25 mg/ml with a mean zone of inhibition of 14.67±0.82, 10.33±0.82, 8.33±0.41, 6.67±0.41, and 4.33±0.82 mm, respectively. While those of aqueous and chloroform fractions showed inhibition zone of 10.50±0.35, 6.67±1.47, 4.67±0.82, 1.67±2.04, and 1.33±1.63 mm for aqueous fraction and 10.33±1.08, 6.67±0.41, 4.33±1.08, and 2.66±1.78 mm, respectively for chloroform fraction. The antimicrobial evaluation expressed as the minimum inhibitory concentration of crude extract showed minimum activity at 25 mg/ml for crude extract and 50 mg/ml for aqueous and chloroform fractions respectively. The various extracts of Securinega virosa (aqueous, chloroform, and crude methanol) were found to possess antibacterial activity on isolated Methicillin-resistant Staphylococcus aureus at varying concentrations, and the crude extract possessed higher activity than the other two extracts.

Key words: *Partitioned fractions, MRSA, Securinega virosa, Antimicrobial*

INTRODUCTION

A major problem is the progressive emergence of multi-drug resistant bacteria and the infectious diseases they cause [1-3]. Clinically significant bacteria like MRSA, vancomycin-resistant enterococci, and Extended-Spectrum β-Lactamase (ESBL) producing *Escherichia coli* are increasing rampantly [4, 5]. Thus, the need for useful and new compounds is ever-growing. Drug resistance in bacteria, the recurrent problems of diseases in persons with organ transplants, the appearance of new life-threatening viruses, and the huge increase in the incidence of fungal infections in the world's population all underscore our inability to face these medical issues [6, 7].
Records show that for centuries, Herbs and medicines derived from herb have played an important role in health and disease management [8]. In most African countries, herbal medicine constitutes 60% of the first line of treatment for children with a high fever from cases of malaria [9]. There is a significant increase for herbal medicinal products due to excessive demand and ''It is estimated that the world's population will be more than

7.5 billion in the next 10 to 15 years''. The southern hemisphere will record more growth, where approximately 80% of the population still relies on a traditional system of medicine based on herbal drugs for primary healthcare [10]. These and many other emphases necessitated the need to carry out this study on the effect of the extract and fractions of *Securinega virosa* on Methicillin-resistant *Staphylococcus aureus* strain.

In addition to causing diseases, bacteria react to the antibiotics used as a treatment by becoming resistant to them. Sooner or later, this natural adaptation process and antimicrobial resistance affects the antibiotics lifespan. The misuse or abuse of antibiotics in recent times has resulted in the commonly observed antibacterial resistance and this has led to ''high mutation of many bacterial strains and spread of resistant bacteria'' [11]. As resistance and virulence increase, its negative impact on society has enormously increased and the damaging effects manifesting themselves across the world. Records show that at least 50,000 lives are being claimed to be antimicrobial-resistant infections yearly across the US and Europe alone, with hundreds of thousands dying in other areas of the world. Recent figures indicate that, by 2050, if proper care is not taken, drug-resistant infections could kill an extra 10 million people globally. World Health Organization is calling on the pharmaceutical industry and governments to work together in fighting drug-resistant infections by an increase in the investment of antibiotic researches to meet global public health needs [12]. New strategies have been implemented to help the healthcare sector coping with antimicrobial resistance and to combat this growing crisis. A promising possible solution for this crisis is the investigation of the antimicrobial activity of the secondary metabolites from natural products. Presently, the exploitation of natural products of plants is now seen as a cheap and rich source of secondary metabolites for antimicrobials. The leaves, stem, and root bark extracts of *Securinega virosa* have been used in the management of different disorders in Nigeria [13, 14]. There is a paucity of scientific information on the potential antimicrobial activity profile of the leaf extract of *Securinega virosa* on MRSA strain. Antimicrobial evaluation is therefore required to predict the effectiveness of this resistance bacterial strain. This study is aimed at evaluating the effect of *Securinega virosa* plant extract and partitioned fractions on Methicillin-resistant *Staphylococcus aureus* (MRSA).

MATERIALS AND METHODS

Digital weighing balance (KERO BLG 300), rotary evaporator, refrigerator (Haier Thermocol®, Model: HRF-250E), and hot air oven (Leader® Model: GP/50/CLAD/250/HYD).

Collection and identification of securingea virosa

The leaves of *Securingea virosa* were gotten from a garden Esan Botanical garden, Edo State. Identification and authentication of specimens were done in the Department of Pharmacognosy and Traditional Medicine by the Head of Department Dr. O.E. Ikpefan. The leaves were washed with water and chopped into smaller pieces, air-dried and powdered using an electric grinder, and then weighed and stored in a clean plastic container in the laboratory before extraction at 25±2°C.

Extraction of the powdered sample of S.virosa

A total amount of 1.2kg of the powdered leaves sample of *S.virosa* was extracted by cold maceration technique extraction of plant sample and phytochemical analysis of S. *virosa* leaves was carried out by the methods described by [15] with little modification.

Sensitivity test of organisms to extract of S. virosa

Preparation of overnight broth culture

The nutrient broth was prepared according to the manufacturer's specification and transferred into bijou bottles and sterilized in an autoclave. After sterilization, the bijou bottles were allowed to cool and Methicillin-resistant *Staphylococcus aureus* (MRSA) bacteria strain obtained from the Pharmaceutical Microbiology laboratory was inoculated into the broth using a sterile wire loop and then incubated at 37 °C for 24 hrs.

Sensitivity test on cefuroxime

Mueller Hinton agar was prepared according to the manufacturer's specification and sterilized in an autoclave. The strains of Methicillin-resistant *Staphylococcus aureus* were spread on the surface of the agar plate. 10 mg of Cefuroxime was diluted using tenfold and two-fold dilution to get 5 µg of Cefuroxime sterile paper disc were inoculated with 5 µg of Cefuroxime solution which was placed on the surface of each culture plate and incubated at 37 °C for 24 hrs. After incubation, the zone of inhibitions was observed following CLSI guidelines [16].

Sensitivity test on crude extract and partitioned fraction of S. virosa

Mueller Hinton agar was prepared according to the manufacturer's specifications and sterilized in an autoclave. It was then allowed to cool and transferred into Petri dishes and left to solidify. On solidification, different bacterial strains were spread on the surface of the agar plate. A two-fold serial dilution of the plant crude extract was done (100 mg, 50 mg, 25 mg, and 12.5 mg and 6.25 mg). Five holes were punched on the agar using a 6 mm cork borer and each hole was labeled according to the different concentrations. The methanol plant extract was transferred into the culture plate according to their labeled concentrations and left for some time to diffuse then it was incubated at 37 °C for 24 hrs. After incubation, zones of inhibition for each concentration were observed [17].

Antibacterial evaluation of crude extracts and partitioned phases of S. virosa expressed as minimum inhibitory concentration (MIC)

The antibacterial evaluation of the crude extracts and different phases of the partitioned extract was carried out and expressed as Minimum Inhibitory Concentration (MIC). The extract and partitioned phase were prepared using two-fold serial dilution which was prepared into about five (5) concentrations (100, 50, 25, 12.5 & 6.25 mg/mL). Five Petri dishes were labeled according to the prepared concentrations of crude extract and partitioned extract. To each of the five plates, the MRSA organisms were labeled on various divisions of each plate. The various concentrations were poured into their respective labeled plates. The sterilized Mueller Hinton agar was poured into the plate also and rocked gently in other to mix properly. The set plate was allowed to solidify. The organism was inoculated on the plate. After which the inoculated plate was incubated at 37 °C for 24 hrs, thereafter the least concentration that inhibited the growth of the MRSA isolate was taken as the Minimum Inhibitory Concentration (MIC) of the crude extract and partitioned phases.

RESULTS AND DISCUSSION

The yield of the extract and fractions

A total of 1.5kg of the powdered sample of *S. virosa* yielded 156g of the extract, implying % 80 g of the partitioned extract gave 42 g (percent) and 23 g (2.21 percent) of the aqueous and chloroform fractions, respectively.

Phytochemical screening of crude extract of S. virosa

Phytochemical screening of the *Securinega virosa* leaves extract and fractions **(Table 1)**.

Table 1. Phytochemical Screening of *Securinega virosa* Leaves Extract and Fractions

Phytochemical Groups	Extract	Fractions	
		Aqueous	**Chloroform**
Alkaloids	++	-	-
Flavonoids	++	+	-
Saponin	+	++	-
Steroids	+	-	++
Tannins	+++	+	-
Cardiac glycosides	++	+	+
Terpenes	++	+	+++
Anthraquinones	+++	++	++

Key: +++: appreciable amount; ++: moderate amount; +: minute amounts; -: not detected

Antimicrobial evaluation of extract and partitioned fractions of S. virosa leaves

A zone of clearance around each well represents the inhibition and the diameter of each zone was measured in millimeters (mm) using a meter rule **(Table 2)**.

Table 2. Zone of Inhibition (mm) of the Extract and Fractions against MRSA

Extract	Conc.mg/ml	1(mm)	2(mm)	3(mm)	Average(mm)
Crude	100	14	16	14	14.67±0.82
	50	9	11	11	10.33±0.82

	25	8	9	8	8.33±0.41
	12.5	6	7	7	6.67±0.41
	6.25	3	5	5	4.33±0.82
Aqueous	100	11	10.5	10	10.50±0.35
	50	9	6	5	6.67±1.47
	25	6	4	4	4.67±0.82
	12.5	5	-	-	1.67±2.04
	6.25	4	-	-	1.33±1.63
Chloroform	100	9	12	10	10.33±1.08
	50	7	6	7	6.67±0.41
	25	3	4	6	4.33±1.08
	12.5	3	-	5	2.66±1.78
	6.25	-	-	-	-
Control (cefuroxime)	-				-

KEY: no inhibition (-)

Antimicrobial Evaluation Expressed as Minimum Inhibitory Concentration (MIC) for Crude Extract and Partitioned Fractions of the Leaf of S. virosa **(Table 3)**.

Table 3. Minimum Inhibitory Concentration for the extract and fractions of *S. virosa* against MRSA

Concentration (mg/ml)	**100**	**50**	**25**	**12.5**	**6.25**
Aqueous	-	-	+	++	++
Crude	-	-	-	+	++
Chloroform	-	-	+	++	++

Key: Growth= +, ++, No growth= -

The result from the qualitative phytochemical analyses showed the presence of alkaloids, saponin, terpenes, tannins, cardiac glycosides, flavonoids, steroids, reducing sugars, and anthraquinones. The finding of this study is in agreement with the report of [18] who reported the presence of secondary metabolites in the methanol extract of the *Securinega virosa* plant. A Similar study was carried out by [19] assessed the phytochemical constituents and antifungal activity of the different solvent extract of *Securinega virosa* with the presence of secondary metabolites found in the various extract of the plant.

Though several works on the sleep-inducing effect [20] and the psychopharmacological activities of *S. virosa* [21], this study evaluates the antimicrobial activity of methanol and different fractions of the extract of *Securinega virosa* leaves. The results obtained from this study show that the leaves of *Securinega virosa* possess antimicrobial activity against Methicillin-resistant *Staphylococcus aureus* (MRSA), and also that the crude extract is most potent among the three extracts that were tested with the highest zone of inhibitions. The aqueous extract only showed inhibition at a concentration of 12.5mg/ml and 6.25mg/ml and chloroform extract showed no inhibition at concentration 6.25mg/ml. The crude showed inhibition at all concentrations. The highest zone of inhibition was seen at a concentration of 100mg/ml in all extracts (crude, aqueous, and chloroform) with diameters 14.67±0.82, 10.50±0.35, and 10.33±0.82mm, respectively.

The antibacterial effect of the extracts on the isolate can be said to be concentration-dependent. Also, the chloroform extract showed no activity at lower concentrations. This finding is not in line with the study of [22] WHO reported a high antimicrobial activity of the chloroform extract of *S. virosa* against test organisms with a diameter zone of inhibition of 13 mm. In the study by Amenu *et al*., [23], ethanol extract of the leaves of *S. virosa* against test organisms showed high antibacterial activity which conforms with the findings from this study.

The control used for this experiment was cefuroxime at a concentration of 5microgram, it showed no activity against the organism (MRSA), this signified that the plant extract is more potent against the organism (MRSA) than the control and also justifies its use in ethnomedicine for the treatment of various infectious diseases. The minimum inhibitory concentration for the crude, aqueous, and chloroform extract was 25mg/mL, 50mg/ml, and 50mg/mL, respectively. The crude had the lowest MIC of 25mg/ml which also showed that it had higher potency against the organism than the other two extracts. This result also once again shows that the antimicrobial effect of

the plant *Securinega virosa* is dependent on concentration. Based on results obtained from this study, it can be deduced that *Securinega virosa* indeed has antimicrobial properties at different concentrations against infectious agents.

CONCLUSION

The various extracts of *Securinega virosa* (aqueous, chloroform, and crude methanol) was found to possess antimicrobial activity on isolated methicillin-resistant *Staphylococcus aureus* at varying concentration, and the crude extract possessed higher activity than the other two extracts due to its synergistic properties which the other extracts did not possess after partitioning. This study supports the traditional use of it for the treatment of ailments associated with bacteria.

Recommendation

Based on this research it is thus recommended that medicinal plants such as *Securinega virosa* should be used as a readily available remedy for bacterial infections in developing countries. However, there is a need for further investigation of the antimicrobial activities of the plant to identify and further isolate the main active compound responsible for its antimicrobial activity. Besides, the safety and toxicity of the plant should be established.

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CONFLICT OF INTEREST: None

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