



ANTIMICROBIAL SUSCEPTIBILITY OF CRUDE EXTRACTS OF *Allium ascalonicum* LINN. (WHOLE PLANT) AND *Gladiolus psittacinus* HOOK (CORM) AGAINST FIVE COMMON PATHOGENS: AN *IN VITRO* INVESTIGATION

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Abstract- *Allium ascalonicum* Linn. and *Gladiolus psittacinus* Hook., are two important plants cultivated across the world for their nutritional as well as medicinal values. In the quest for a more natural bioactive substance to check the rising incidence of antimicrobial resistance amongst pathogens, these two plants were in this study, screened for activity against five common pathogens; namely, *Staphylococcus aureus* ATCC 29213, *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 35657, and *Candida albicans* ATCC 90029. Phytochemical screening carried out on the extracts showed the presence of secondary metabolites like saponins, cardiac glycoside, alkaloids and flavonoids in both plant samples but only *Allium ascalonicum* yielded essential oil, while carbohydrate and tannins were detected in *Gladiolus psittacinus*. While the crude extracts of *Gladiolus psittacinus* did not show any inhibitory potential against any of the tested organisms, three pathogens were susceptible to *Allium ascalonicum*, with minimum inhibitory concentration (MIC) of 100 mg/mL. Bactericidal kinetics studies on the three pathogens showed that within the exposure time of 4 hours, the organisms were killed (69% and 95% at 2 × MIC and 4 × MIC, respectively), a result which compared well with the positive control Gentamicin. The result of this study while agreeing with earlier reports on the non-activity of *Gladiolus* corms against *Candida albicans* contradicts the same report on the activity of the crude extract of this plant against *Pseudomonas aeruginosa*. The significant inhibitory potential of the crude methanolic extract of *Allium ascalonicum* against food borne pathogens such as *Staphylococcus aureus*, *Escherichia coli* and nosocomial pathogen such as *Klebsiella pneumoniae*, suggests that it can be explored as a natural alternative to chemical preservatives in food industries, and in the formulation of drugs against nosocomial infections.

Keywords- *Allium ascalonicum* Linn., *Gladiolus psittacinus* Hook., *in vitro* sensitivity, Bactericidal kinetics, phytochemical screening, secondary metabolites, nosocomial infection, pathogen

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Introduction

The menace of common pathogens like *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Candida albicans*, *Klebsiella pneumoniae* etc., has remained a source of concern to healthcare providers. *Staphylococcus aureus* is a virulent pathogen that is currently the most common cause of infection in hospitalized patients [1]. The increase in the resistance of this pathogen to antimicrobial agents, coupled with its increasing prevalence as a nosocomial pathogen, is of major concern. It causes a wide range of suppurative infection, as well as food poisoning and toxic shock syndrome [2].

Pseudomonas aeruginosa is another very notorious pathogen. It is responsible for many nosocomial infections, and causes life-threatening diseases in immunocompromised persons; and chronic infections in cystic fibrosis patients [3]. It is an opportunistic human pathogen and that of plants [4]. One most worrisome characteristics of *P. aeruginosa*, is its low antibiotic susceptibility, which is attribut-

able to a concentrated action of multidrug efflux pumps with chromosomally-encoded antibiotic resistance genes [5]. *Escherichia coli* are commonly found in the lower intestine of warm-blooded organisms, most of which are harmless but some serotypes are pathogenic and cause serious food-poisoning in humans. They are responsible for occasional product-recalls [3,6]. *Candida albicans*, a diploid fungus that grows both as yeast and filamentous cells, are the causal agents of opportunistic oral and genital infections in human [7,8]. In recent years, *Klebsiella* have become important pathogen in nosocomial infections; and *K. pneumoniae* is clinically the most important member of the *Klebsiella* genus of Enterobacteriaceae worrisomely, new antibiotic-resistant strains of *K. pneumoniae* are appearing, and it is increasingly found as a nosocomial infection [9].

Allium ascalonicum is an herb reported for its high medicinal values. Its properties are considered stomachic, tonic, anthelmintic, antispasmodic, aphrodisiac, diuretic, expectorant, febrifuge and hypo-

tensive among others [10]. A member of the onion family, it is reported to have shown therapeutic potential against *Helicobacter pylori*, [11]. Its ethanolic and aqueous extracts have also been reported to inhibit the growth of *Mycobacterium tuberculosis* [12,13]. Partially purified extract of *A. ascalonicum* with sodium benzoate reportedly, synergistically inhibited all food-borne fungi, suggesting its use as food preservative, as to minimize the use of chemical preservatives [14,15].

Gladiolus is an African medicinal plant recorded in the human pharmacopoeia. It is recorded (under several of its synonyms) as being used in southern Africa in the treatment of variety of ailments, including diarrhoea and colds [16]. In parts of West Africa, *Gladiolus* is used in preparations to cure both constipation and severe dysentery. In Ghana, Nigeria, Cameroon and Botswana; *Gladiolus* corms are used in food and in ethno-medicine for treating infections [17]. In Ghana and Igalaland (North-central Nigeria), *G. psittacinus* is actually cultivated on a small scale [18], while in south-western Nigeria, the corms called 'Baka' are used in treating gonorrhea, dysentery, and other infectious conditions.

Materials and Methods

Collection of Plant Samples and Preparation of Extracts

The plants samples- *Allium ascalonicum* Linn. and *Gladiolus psittacinus* Hook. were collected from Ogbomoso area of Oyo state Nigeria and authenticated at the herbarium in the Department of Botany, University of Ibadan, Ibadan, Nigeria. Voucher specimens were deposited for reference purposes, with the voucher numbers UIH-22337 and UIH-22336 for *Allium ascalonicum* Linn. and *Gladiolus psittacinus* Hook respectively. The *A. ascalonicum* plants were properly washed and air-dried while the corms of *G. psittacinus* were chopped into bits and sun-dried. They were later pulverized and packed into the Soxhlet extractor chamber. Continuous extraction process, using the *n*-hexane and methanol as solvents of choice, for 18 to 24 hours, was applied. The filtered extracts were concentrated using rotary evaporator; and dried. They were stored in a refrigerator until needed.

Various concentrations of the extracts viz: 200 mg/mL, 100 mg/mL, 50 mg/mL, 25 mg/mL were prepared and used in this study.

The Test-Microorganisms

These include one Gram-positive organism, *Staphylococcus aureus* ATCC 29213; three Gram-negative organisms, *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 35657, and one fungal species, *Candida albicans* ATCC 90029. They were grown in the appropriate broth culture; Tryptic Soy broth (TSB) and subcultured overnight prior to the sensitivity testing to keep them in the log phase.

Equipment and Other Items

Autoclave (Gallenkamp), rotary evaporator, hot-air-oven, electronic weighing balance (mettler), Soxhlet extractor, water bath (Gallenamp), various calibrated glassware, incubator (Gallenkamp), refrigerator, glass Petri dishes, pipettes, corkborer; media snutrient agar, Tryptic Soy broth, Mueller-Hinton agar, nutrient broth and Sabouraud dextrose agar.

Phytochemical Screening

The extracts were chemically analyzed for the presence of secondary metabolites such as anthraquinones, cardiac glycosides, flavo-

noids, saponins, tannins, alkaloids as well as essential oil. This was done using the methods and techniques described by [19,20].

Antimicrobial Activity

Sterile plates were seeded with 1:100 dilution of fresh overnight culture of the test organisms [21], by introducing 0.2 mL of the organisms from the dilution into 20ml of molten Mueller-Hinton agar (for bacteria) and Sabouraud dextrose agar (for fungal species) kept at 45°C. The mixture was properly blended in a universal bottle before pouring into the plates and allowed to set. A sterile cork borer of 8 mm diameter was used to make uniform and equidistant wells on the seeded plates. One hundred microlitre (100 µL) of the resuspended extracts was placed in the wells using sterile pipettes, the positive and negative controls were also placed in their respective wells. The plates were allowed to stand on the bench for an hour to ensure proper diffusion before incubation. After 24-hour incubation at 37°C for bacteria species, and 2 to 3 days at 25°C for fungal species, the plates were observed for zones of inhibition, which were measured to the nearest millimeter (mm) with a standard transparent meter rule. All tests were performed in duplicates.

Determination of Minimum Inhibitory Concentration (MIC)

The agar dilution technique [22] was adopted to determine the MIC for susceptible organisms. The resuspended extracts were prepared to final concentrations of 200 mg/mL, 100 mg/mL, 50 mg/mL, 25 mg/mL and 12.5 mg/mL. 1 mL of each concentration was introduced into 19 mL of molten Mueller-Hinton agar maintained at 45°C. The properly blended mixture was poured into sterile Petri dishes and allowed to set before removal of moisture in a sterile dryer. The test organisms from a fresh overnight broth culture were streaked on the surface of the dried agar in plates. The plates were incubated at 37°C for 24 hours after which they were examined for growth, recording the lowest concentration that prevented the growth of the organisms as their minimum inhibitory concentration. All the tests were performed in duplicates.

Extracts' Kill-Kinetics

Concentrations of extracts corresponding to that of MIC, 2xMIC, 4xMIC, 8xMIC were used, by a modification of methods described by [21]. One hundred microlitre (100 µL) of fresh overnight culture of the susceptible organisms, maintained in a sustained log phase of growth, was added into 3.9 mL of Tryptic soy broth, containing 1 mL of the extract, to give a 5-fold dilution. This formed the stock for each concentration. 0.1 mL of the stock was aseptically withdrawn to carry out serial dilution, and 0.1 mL of the final dilution, plated out on a solidified, sterile Mueller-Hinton agar. A sterile glass rod was used to spread the inoculums. The withdrawal from the stock was strictly done at time intervals of 0 hour, 0.5 hour 1.0 hours, 2.0 hours, 4.0 hours, 6 hours, and 24 hours, for the various concentrations; and this also ran simultaneously with the drug-positive control and organism-negative control. The plates were incubated at 37°C for 24 to 48 hours, after which the number of colonies on each plate was counted, and the colony forming unit (CFU), calculated taking into account the final volume inoculated and the dilution factor. The rate of kill was determined from the percentage survival of the organisms. This was presented on a semi-logarithm graph. All tests were performed in duplicates.

Results

The percentage yield and macroscopic features of the plants ex-

tracts are presented in [Table-1] while the results of the phytochemical screening of samples are as shown in [Table-2]. [Table-3] and [Table-4] show the results of the antimicrobial sensitivity testing as well as the minimum inhibitory concentration of the active extract on

the test organisms. The bactericidal activities of bioactive methanol extract on test pathogens are depicted in [Fig-1], [Fig-2] and [Fig-3] showing the percentage kill of the organism at different exposure time.

Table 1- Percentage yield and macroscopic analysis of *n*-Hexane and Methanol extracts of *Allium ascalonicum* Linn. and *Gladiolus psittacinus* Hook

Plant species	Weight of Samples (g)	% yield		Macroscopic Analysis	
		Hexane fraction	Methanol fraction	Hexane fraction	Methanol fraction
<i>Allium ascalonicum</i> (whole plant)	543	1.98	17.7	Greenish-yellow (solid)	Dark-brown (paste)
<i>Gladiolus psittacinus</i> (Dried corn)	810.8	1.86	19.59	Pale-yellow (viscous liquid)	Brown (paste)

Table 2- Phytochemical screening of the extracts of *Allium ascalonicum* Linn. and *Gladiolus psittacinus* Hook.

Phytochemicals	<i>A. ascalonicum</i>	<i>G. psittacinus</i>
Alkaloids	++	++
Antraquinones	-	-
Cardiac glycosides	++	++
Carbohydrates	-	++
Flavonoids	++	++
Saponins	+	++
Tannins	-	++
Essential oil	++	-
Terpenoids	-	-

Note: + = present in moderate concentration

Table 3- Antimicrobial Sensitivity of the five common Pathogens to the extracts of *Allium ascalonicum* Linn. and *Gladiolus psittacinus* Hook. at 100 mg/mL. Zone of inhibition (mm) \pm SEM

Organism	<i>A. ascalonicum</i>		<i>G. psittacinus</i>		Gentamicin (80 μ g/ml)
	<i>n</i> -Hexane	Methanol	<i>n</i> -Hexane	Methanol	
<i>S. aureus</i> ATCC 29213	NA	16 \pm 0.20	NA	NA	20 \pm 0.40
<i>P. aeruginosa</i> ATCC27853	NA	NA	NA	NA	NA
<i>E. coli</i> ATCC25922	NA	16 \pm 0.25	NA	NA	21 \pm 0.50
<i>K. pneumonia</i> ATCC35657	NA	17 \pm 0.05	NA	NA	21 \pm 0.20
<i>C. albicans</i> ATCC90029	NA	NA	NA	NA	NA

Note: Diameter of cork borer = 8mm; NA = No Activity; Results are average of duplicate experiments

Table 4- Minimum Inhibitory Concentration (MIC) of methanol extract of *Allium ascalonicum* Linn. on the test pathogens

Extracts (mg/mL)	<i>S. aureus</i> ATCC 29213	<i>P. aeruginosa</i> ATCC 27853	<i>E. coli</i> ATCC 25922	<i>K. pneumonia</i> ATCC 35657	<i>C. albicans</i> ATCC 90029
<i>A. ascalonicum</i> (mg/mL)	100	ND	100	100	ND
Gentamicin (μ g/mL)	80	ND	80	80	ND

Note: ND = Not determined, Results are average of duplicate experiments

Discussion and Conclusion

In the quest for plants with the right composition and combination of phytochemical compounds as possible antidote to the problems of drug-resistance amongst pathogenic microorganisms, this study investigated susceptibility of five common pathogens to the crude *n*-hexane and methanol extracts of *Allium ascalonicum* (whole plant) and *Gladiolus psittacinus* (dried corn). The yield of the methanol fraction of the extract was more than the yield of *n*-hexane fraction of the extract [Table-1]. The non-polar nature of *n*-hexane must have contributed to its low percentage yield as shown in [Table-1] because during extraction process, solvents diffuse into the solid plant material and solubilise compounds of similar polarity. This is known to affect the quantity and composition of secondary metabolites of the extract [23]. The qualitative phytochemical analysis of the plant extracts revealed the presence of secondary metabolites such as alkaloids, flavonoids, saponins, and cardiac glycosides, in both plant samples but only *G. psittacinus* yielded tannins and carbohydrate, while essential oil was found in *A. ascalonicum*. Anthraquinones and terpenoids were not detected [Table-2].

The lack of inhibitory potential observed in the methanol extract of *G. psittacinus* against the common pathogens may not necessarily mean the absence of bioactive constituents; but may be due to its poor diffusion rate as observed in this study [Table-3], occasioned by the high molecular weight of the extract. In contrast, the methanol extract of *A. ascalonicum* showed significant inhibitory potential against three of the investigated pathogens. *S. aureus* ATCC 29213, *E. coli* ATCC 25922, *K. pneumoniae* ATCC 35657 [Table-3] due to the presence and right combination of the secondary metabolites, as well as the high diffusion rate and also, the presence of essential oil which being lipophilic in nature, makes permeability to

cellular membrane easy [24]. The antimicrobial properties exhibited by the methanol extract of *A. ascalonicum* could be traced to the presence of alkaloids, flavonoids, saponins, and cardiac glycosides and most especially essential oil. These phytochemical compounds are biologically active and thus aid the antimicrobial activities of *A. ascalonicum*. Phytochemicals exert antimicrobial activity through different mechanisms.

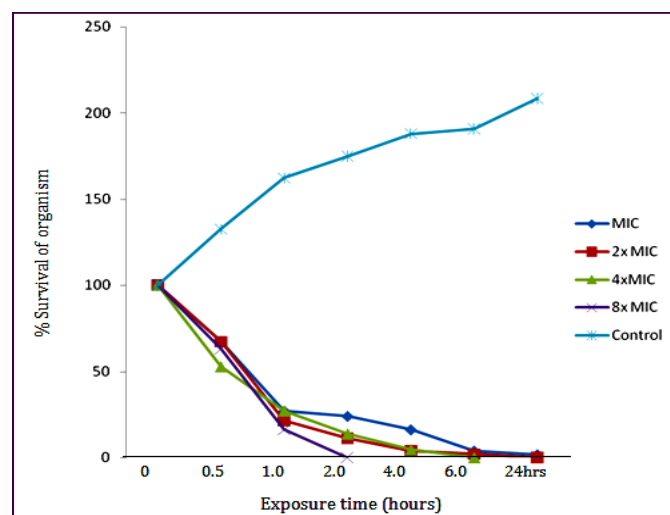


Fig. 1- Kinetics of bactericidal activities of methanol extract of *A. ascalonicum* on *S. aureus* ATCC 29113, showing the rate of kill of the organism by the extract at different exposure time.

The resistance or low antibiotics susceptibility of *P. aeruginosa* has been attributed to a concerted action of multidrug efflux pumps with chromosomally-encoded antibiotic resistant genes [5]; and unusual

ly large number of genes for catabolism, nutrient transport, efflux of organic molecules and metabolic regulations [25]. Also *C. albicans* which showed resistance to the plant extracts in this study as shown in [Table-3] have been reported to have several mechanisms of antimicrobial resistance [26-28].

In agreement with its MIC value of 100 mg/mL as shown in [Table-4], the bactericidal kinetics showed that at concentrations equivalent to 2 x MIC and 4 x MIC, the susceptible organisms were killed (69% kill and 95% kill respectively), within 4 hours exposure time [Fig-1] to [Fig-3]. The killing was both dose and time dependent as shown in the graph. This compared well with the drug-positive control which killed the organisms at about the same contact time though at a much lower concentration [Fig-1] to [Fig-3]. The bactericidal activity exhibited by the drug control is expected since it is a pure drug unlike the methanol extract which may have a lot components acting together at the same time; the action/activity which may be synergistic, additional or even antagonistic.

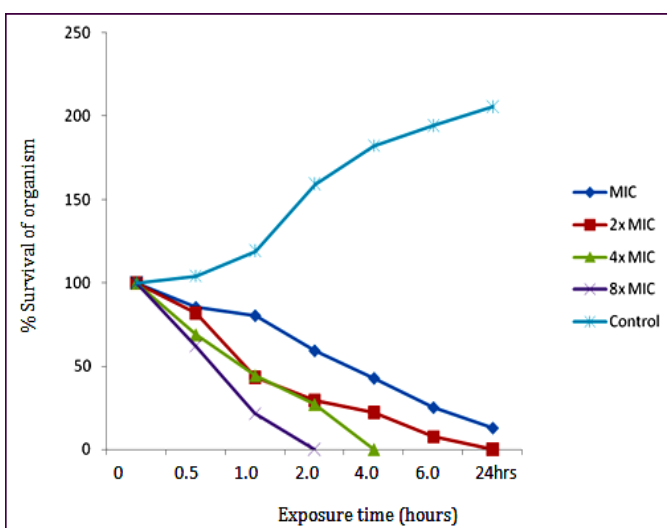


Fig. 2- Kinetics of bactericidal activities of methanol extract of *Allium ascalonicum* on *Escherichia coli* ATCC 25922, showing rate of kill of the organism by the extract at different exposure time.

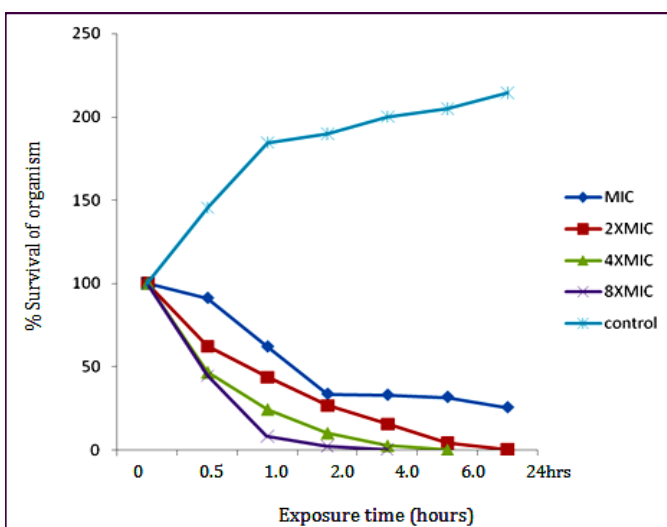


Fig. 3- Kinetics of bactericidal activities of methanol extract of *Allium ascalonicum* on *Klebsiella pneumoniae* ATCC 35657, showing rate of kill of the organism by the extract at different exposure time.

Conclusion

In conclusion, while the crude extract of *G. psittacinus* did not show inhibitory potential against any of the common pathogens investigated, further purification of the bioactive constituents may yield a better result. The significant inhibitory potential displayed by the methanol extract of *A. ascalonicum* against common food-borne pathogens like *S. aureus*, *E. coli*, and nosocomial pathogen like *K. pneumoniae* suggests that it can be used as a possible natural preservative in food industries as to reduce the risks involved in the use of chemical preservatives and also in formulation of drugs against infections involving nosocomial pathogens.

Conflicts of Interest: None declared.

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