JOURNAL OF BIOPESTICIDES AND AGRICULTURE
2465-7115 (Online); 2465-7123 (Print)
http://www.biopjournal.com

IN VITRO BIOACTIVITY OF *Allium sativum* and *Allium cepa* EXTRACTS AGAINST SOME WOUND PATHOGENS IN CALABAR, CROSS RIVER STATE, NIGERIA.

Markson, A. A[1], *Akpan, J. B[1], Anwan, E. E.[2], Umana, E. J.[1], Udo, S. E. and Akwaji, P. I[1].
1. Department of Botany, University of Calabar, Calabar, Cross River State, Nigeria.
2. Department of Medical Microbiology and Parasitology, University of Calabar Teaching Hospital, Calabar, Cross River State, Nigeria.
*Corresponding author's e-mail: julietakpanb@gmail.com

ABSTRACT

Antibacterial susceptibility potential of *Allium sativum* and *Allium cepa* extracts were tested on four wound pathogens: *staphylococcus aureus*, *pseudomonas aeruginosa*, *Escherichia coli* and *klebsiella spp*. Phytochemical screening of the crude extracts revealed the presence of some bioactive compounds that have been associated with antimicrobial activities. Both garlic and onions extracts (raw, hot and cold water extract) showed good antibacterial activities against most of the pathogens. However, garlic was the most effective, even at the different concentration levels. The raw extracts gave the widest zones of inhibition; this was followed by the performance of hot water extracts. The least zones of inhibitions were obtained from cold water extracts. The largest mean inhibition zone diameters were recorded with E. coli and Pseudomonas with a peak mean value of 38.0 mm and 39.0 mm respectively both obtained in the raw extract of garlic, while the least was observed in *klebsiella spp,* with a mean value of 10.0 mm with onion extracts at 20 mg/ml. This study has revealed the potentials of garlic and onions in the management of wound infection.

Key words: Phytochemical screening, antibacterial, *Allium sativum* and *Allium cepa* extracts.

1. INTRODUCTION

A wound is a break in the epithelial integrity of skin or tissue which may be caused by physical, chemical or microbial agents (Ogwang *et al*, 2011). When such breakage occurs in the skin, opportunistic microorganisms usually invade and multiply in the developing cavities. Such invasion and colonization of wounds by pathogens has been shown to delay healing process and may lead to infectious condition. Research reveals that eradication of these pathogens results in rapid wound healing (Aubakar, 2009). Some of the bacteria commonly isolated in wound infections include *Pseudomonas*, *Staphylococci*, *Escherichia coli* and *Klebsiela* amongst others. However, these bacteria have been reported to have greater resistance and virulent capabilities including the formation of biofilms on colonized surfaces (Aubakar, 2009). Therefore, concern exists worldwide about the threat posed to human health by antibiotic resistance in these microbial pathogens. The fact that microorganisms have gradually developed resistance to drugs has created a situation where some of the common antimicrobial agents are becoming less effective. This development has led to increasing interests and dependence on herbal remedies in many parts of the world. In many cases, the herbal practices have been incorporated into existing orthodox medical procedures (Apata 1979). This increasing interest also stems from the fact that natural remedies are body friendly. They do not kill friendly micro flora or make one more susceptible to future infections as orthodox antibiotics do (Weli, 1990). Numerous plants and herbs across the globe exhibit antimicrobial and other medicinal properties. These plants are usually referred to as medicinal plants. According to Akinpelu (1999), medicinal plants are plants in which one or more of their organs contains substances that can be use for therapeutic processes or are precursors of the synthesis of useful drugs. Medicinal plants may also refer to plants which contain one or more active constituent, capable of preventing or treating diseases or disorders. Although many plants around the world fit into this definition, this study screened for phytochemical constituents and examined the antimicrobial potency of two of such plants – *Allium cepa* (onions) and *Allium sativum* (garlic) against some clinical wound pathogen - *staphylococcus aureus*, *pseudomonas aeruginosa*, *Escherichia coli* and *klebsiella spp.*

2. MATERIALS AND METHODS

2.1 Collection of plant materials

The bulbs of onions and garlic used for this study were purchased from the local markets in Calabar, Cross River State. The organisms used were isolated from 16 wound swab samples from patients in the microbiology laboratory Department of the University of Calabar Teaching Hospital, Calabar (UCTH), Cross River State, Nigeria. Further biochemical tests were carried out to confirm the identity of the organisms. These tests included gram staining, catalase, coagulase, oxidase, motility and carbohydrate fermentation tests. The pure cultures of the organisms were sub-cultured onto nutrient agar slants and preserved in the laboratory refrigerator at 4^{o}c until required for use.

2.2 Preparation of plants extracts

The two test plants were extracted in three ways namely: hot water extracts, cold water extracts and raw extracts. In each of the extracts, both onions (*Allium cepa*) and garlic (*Allium sativum*) were washed with sterile distilled water and the outer coverings were manually removed. The onions and garlic were washed again and allowed to air-dry. The dried onions and garlic were then chopped into very fine pieces and 20 g, 40 g, 60 g, and 80 g, of the chopped onions and garlic were aseptically weighed and put into separate labelled sterile containers. For cold water extracts, 100 ml of sterile cold water was poured into each container. For hot water extracts, 100 ml of hot water at 100^{o}C was poured into each container. The mixture was allowed to stand for 12 hours, after which the liquid was filtered using sterile muslin cloth, into labeled sterile containers. Raw or undiluted extracts were obtained by aseptically blending well washed and peeled onions or garlic. Then 2ml of the extracts were filtered into labeled sterile containers.

2.3 Preparation of antibiotics discs from plant extracts

The sensitivity discs were prepared from Whatman No1 filter paper. The filter papers were cut into discs of about 6mm in diameter, labeled and sterilized by autoclaving at 121^{o}C for 15 minutes. The discs were then soaked in the appropriately prepared plant extracts and allowed to dry under room temperature. When the discs were stored for use in correctly labeled sterile Petri-dishes corresponding to the different extracts.

2.4 Antimicrobial sensitivity test

Antimicrobial sensitivity test was carried out using disc diffusion method. The test microorganisms were transferred from nutrient broth to sterile Potato Dextrose Agar (PDA) plates with the help of sterile cotton swabs. Using a flamed-sterilized forceps, the prepared antibiotics (discs from plant extracts) were aseptically placed over the agar plates seeded with the test microorganisms. Plates were incubated in an upright position at 37°C for 24 hours. After 24 hours, the diameter of zone of inhibition was measured. The antibacterial activity was expressed in terms of the diameter of zone of inhibition. Less than 9mm zone was considered as inactive; 9-12mm as partially active; while13-18mm as active and more than 18mm as very active (Junior and Zanil, 2000).

2.5 Phytochemical analysis of plant extracts

The methods described by Harborne (1973) and Trease and Evans (1989) were used to screen for the presence of the phytoconstituents. The dried extracts were first reconstituted in the respective solvents used for their extraction and then tested by standard phytochemical methods for the presence of some bioactive compounds - alkaloids, saponins, flavonoids, anthroquinones, phlobatannins, tannins, cardiac glycosides.

2.6 Statistical analysis

Data collected were subjected to analysis of variance using SPSS and means were separated using LSD at 0.05 probability level.

3. RESULTS

The antibacterial activity of the extracts (raw, hot or cold) of garlic and onions was evaluated by measuring the sizes of the zones of inhibition produced by the extracts against the test bacteria (*staphylococcus aureus*, *pseudomonas* *aeruginosa*, *Escherichia coli* and *klebsiella sp.*) as shown in Table 1. The susceptibility of the bacteria to the extracts, varied according to microorganism, type and concentration of extracts. Generally, the largest mean inhibition zone diameters were recorded with *E. coli* and *Pseudomonas* with peak mean values of 38 mm and 39 mm respectively both obtained in the raw extract of garlic, while the least was observed in *klebsiella spp,* with a mean value of 10.0 mm with onion extracts at 20 mg/ml. Based on the diameters of zones of growth inhibition, the raw extracts of both garlic and onions were more potent when compared to the diluted variants, although all levels of dilutions [even the lowest level (20 mg/ml)] recorded growth inhibitions. Nejad (2014) reported that dilute solutions of garlic could completely inhibit the growth of *S. aureus* at the concentration of more than 7.50 mg/ml. This may be attributed to the action of allicin (Hovana *et al*, 2011). Allicin is a sulphur-containing, biologically active compound that exhibits its antimicrobial activity mainly by immediate and total inhibition of RNA synthesis, although DNA and protein syntheses are also partially inhibited, suggesting that RNA is the primary target of allicin action Deresse (2010). The hot water extracts (both garlic and onions) gave wider zones of inhibition compared to those of cold water extracts (Table 2). This implies that hot water was better as extraction solvent compared to cold. Perhaps the oil component of garlic and onions made available by the raised temperature of water offered the antimicrobial potency in this case. This can be resolved when the bioactive compounds have been isolated and the molar activity of the purified form determined (Mbuh *et al*, 2008; Abubakar 2009). Hannan *et al*, 2010 associated the antimicrobial activity of onions to the presence of its volatile oils and other component. Irrespective of solvent of extraction and level of concentration, garlic extract performed better than onions extracts (Table 2), this is likely due to the presence of alliin (a more potent compound) as a major component in garlic (Lanzotti, 2006).

Bacterial growth inhibition may also be as a result of the presence of other phytoconstituents identified in garlic and onions. Such phytochemical include alkaloids, flavonoids, saponins, anthroquines, tannins and cardiac glycosides (Table 3). These phytochemicals have been reported by several researchers to exhibit antimicrobial properties. Eltaweel (2013) reported that the antibacterial activity of onion juice has been shown to have broad spectrum antibacterial activity. Alkaloids, saponins, flavonoids and polyphenols have been observed to exhibit antibacterial properties against some common clinal bacterial isolates (Otunola *et al.*, 2010; Abubakar, 2009, Akinyemi *et al*, 2006). Akrayi and Abdulrahman (2013) explained that the antimicrobial effect of tannins may be related to their ability to inactivate microbial adhesins, enzymes and cell envelope transport proteins.

4. CONCLUSION

The sensitivity of the isolates to the test plant extracts increased with increase in concentration and the raw extracts were most effective. Hot water was better as extracting solvent compared to cold water. Garlic extracts were more effective as antimicrobials against the wound pathogens than onions. The fact that antimicrobial resistance was not seen to occur in garlic and onions prove these plants to be very effective antimicrobial. Garlic was shown to be active against organisms that have been reported to exhibit resistance to certain antibiotics, (Sivam, 1998, Eja *et al*, 2007). The result of this study emphasizes the potency of *Allium sativum* (garlic) and Allium cepa (onions) effective natural therapy for the treatment of wound infections.

REFERENCES

Abubakar, E. M. (2009). The use of *Psidium guajava* Linn. in treating wound, skin and soft tissues infections. *Scientific Research and Essay,* 4 (6), 605-611.

Akinpelu, D. A. (1999). Antimicrobial activity of *Veronina amygdalina* leaves. *Journal for the Study of Medicinal Plants* 70, 432-435.

Akinyemi, k. O., Oluwa, O. K. and Omomigbehin, E. O. (2006) Antimicrobial activity of crude extracts of three medicinal plants used in south-west Nigerian folk medicine on some food borne bacterial

pathogens. *African Journal of Traditional*, *Complementary and Alternative Medicines, 3* (4), 13 – 22.
Akrayi, A. F. S. and Abdulrahman, Z. F. A. (2013) Evaluation of the antibacterial efficacy and the phytochemical analysis of some plant extracts against human pathogenic bacteria. *JPCS* (7), 29 – 39.
Apata, L. (1979). Practice of Herbalism in Nigeria. University of Ife Press.
Deresse, D. (2010). Antibacterial Effect of Garlic (*Allium sativum*) on *Staphylococcu aureus*: An *in vitro* Study. *Asian Journal of Medical Sciences* 2(2), 62-65.
Eja, M. E., Asikong, B. E., Abriba, C., Arikpo, G. E., Anwan, E. E. and Enyi-idoh, K. H. (2007). A comparative assessment of the antimicrobial effects of garlic (*Allium sativum*) and antibiotics on diarrheagenic organisms. *Southeast Asian Journal of Tropical Medicine and Public Health,* 38, 343-348.
Eltaweel, M. (2013). Assessment of Antimicrobial Activity of Onion Extract (*Allium cepa*) on *Staphylococcus aureus; in vitro* study. International Conference on Chemical, Agricultural and Medical Sciences (CAMS-2013) Dec. 29-30, 2013 Kuala Lumpur (Malaysia) Pp 60 - 62.
Hannan, A., Humayun, T., Hussain, M. B., Yasir, M. and Sikandar, S. (2010). *In vitro* antibacterial activity of onion (*Allium cepa*) against clinical isolates of *vibrio cholerae. J Ayub Med Coll Abbottabad,* 22 (2), 160 -163.
Hovana, E. K., James, U. S., James, E., Egbobor, E. M., Egba, A. G., Eta, E. S. and Nwakaku, O. A. (2011). Antibacterial and Phytochemical Studies of *Allium Sativum*. *New York Science Journal,* 4(4), 123 - 128.
Lanzotti, V. (2006). The analysis of onion and garlic. *Journal of Chromatography A,* 1112: 3–22.
Nejad, A. S. M., Shabani, S., Bayat, M. and Hosseini, S. E. (2014). Antibacterial Effect of Garlic Aqueous Extract on *Staphylococcus aureus* in Hamburger. *Jundishapur Journal of Microbiology*; 7(11), e13134.
Otunola1, G. A., Oloyede, O. B., Oladiji, A. T. and Afolayan, A. J. (2010). Comparative analysis of the chemical composition of three spices – *Allium sativum* L. *Zingiber officinale* Rosc. and *Capsicum frutescens* L. commonly consumed in Nigeria. *African Journal of Biotechnology,* 9 (41), 6927-6931.
Owang, P. E., Nyafuono, J., Agwaya, M., Omujal, F., Tumusiime, H. R. and Kyakulaga, A. H. (2011). Infections. Preclinical efficacy and safty of herbal formulation for management of wounds. *African Health Sciences,* 11(3): 524 -529.
Sivam, G. P. (1998) Protection against Helicobacter pylori and other bacterial infections by garlic (Abstract). Newport Beach CA. The conference of the Recent Advances in the Nutritional Benefits Accompanying the Use of Garlic as a Supplement 15 -17.
Trease, G. E. and Evans, W. C. (1989). Pharmacognosy. Lodon, Bailliere Tinai Ltd.
Weli, A. (1990). Natural Health, National Medicine (Boston: Houghton-Mifflin company), P 240.

Table 1: Influence of treatment levels on the zones of inhibition of the bacteria

Plants	Conc.	Zone of Inhibition (mm)			
		E. coli	*Kliebsiella*	*Pseudomonas*	*Staphylococcus*
Garlic	0	38.0	21.0	39.0	18.0
	20	21.3	14.3	26.8	13.3
	40	23.0	15.0	29.0	13.8
	60	27.3	15.5	30.5	15.0
	80	29.3	16.7	32.3	15.8
Onions	0	33.5	13.0	26.0	15.5
	20	22.0	10.0	17.0	11.8
	40	22.8	10.0	18.7	12.3
	60	27.0	10.8	19.5	13.0
	80	28.3	11.3	22.0	13.5
LSD = 0.10					

Values are means of 3 replicates.

Table 2: Influence of extracts on the zones of inhibition of the bacteria

Extracts	Plants	Zone of Inhibition (mm)			
		E. coli	*Kliebsiella*	*Pseudomonas*	*Staphylococcus*
Cold water	Garlic	26.6	15.4	29.0	14.7
	Onions	25.3	10.4	19.7	12.6
Hot water	Garlic	28.9	17.6	34.0	15.6
	Onions	28.1	11.6	21.6	13.8
LSD = 0.15					

Table 3: Phytochemical Constituents of *Allium sativum* and *Allium cepa*

Constituent	*Allium sativum*	*Allium cepa*
Alkaloids	+	+
Tannins	+	+
Saponin	+	+
Flavonoids	+	+
Anthraquinones	+	+
Cardiac glycosides	+	+
Phlobatanins	-	-

+ Present - Absent

****