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COMPARATIVE ASSESSMENT OF ANTIMICROBIAL ACTIVITIES OF *Allium cepa* (ONIONS) EXTRACTS

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ABSTRACT

The evolution of resistivity by microorganisms is a worldwide public health contest that renders antimicrobial agents impotent, leading to an upsurge in diseases and mortality in man and livestock. The current study was designed to ascertain the antimicrobial activities of *Allium cepa* (onions) extracts. The antimicrobial activities of the onion extracts on the trial organisms, including both gram-positive and -negative bacteria, were carried out using the agar well diffusion method. The tube macro-dilution method determined the extracts' MIC (mg/mL). We investigated the MBC and MFC of the extracts. The antimicrobial assay proved that; the microorganisms tested were sensitive at 50mg/mL but resistant at 3.125mg/mL. From the MIC results, the increasing order of activity of the extracts was *Staphylococcus epidermidis* (MIC 3.125mg/mL), *Pseudomonas aeruginosa*, *Escherichia coli*, and *Salmonella typhimurium* (MIC 6.25mg/mL), *Candida albicans* and *Staphylococcus aureus* (MIC 12.5mg/mL) and Klebsiella pneumoniae (25mg/mL). The MBC results proved that the onion extracts (50mg/mL) were bactericidal except for *Staphylococcus aureus* and fungistatic. Comparatively, at a 5% alpha level, pronounced differences were observed between the zones of inhibition and the solvent extracts by cold and hot water extract on the experimental organisms. However, no significant disparity was observed between the effect of aqueous and solvent extracts. Hence, the study recommends using onions, natural herbal medicine, and antimicrobial agents.

Keywords: *Allium cepa*, Antimicrobial assay, MIC, MBC, MFC

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1. INTRODUCTION

The rate at which microorganisms develop resistance to antibiotics has increased tremendously and is becoming a universal health challenge (Gerber 2010). *Allium cepa*, popularly known as onion has been one of the ancient vegetables in history (Gautam et al. 1997; Mehta 2017; Semerci et al. 2020; Fredotović et al. 2021). It is a condensed rhizome, plump, in shape, having a thin, dry sheath which appears either red or white in color (Azu et al. 2007; Gomaa 2017; Sarvinehbaghi et al. 2021). Its constituents include protein, fats carbohydrate, minerals, and Vitamins (Vamshi et al. 2010). It also has a good number of amino acids, necessary for virtually all biological purposes (Yasmin et al. 2018).

Onion has been employed in traditional medicine for the treatment of bacterial and fungal infections (Edeoga and Erratodo 2000; Banerjee et al. 2003; Benkeblia 2004; Wetli et al. 2004; Krstin et al. 2018; Oghenochuko et al. 2020). It can also be used as a preserving agent in foods (Pszczola 2002; Ye et al. 2013; Farag et al. 2017; Teshika et al. 2019). The active principles in onions extracts affect micro-organisms in different ways. These organisms also differ in their degree of susceptibility to these bioactive principles (Tapsell et al. 2006; Gorinstain et al. 2010; Giovannini et al. 2016; Induja and Geetha 2018; Gupta et al. 2021). Onions are shown to be active against cough and sore throat (Ye et al. 2013), common cold, diabetes, osteoporosis, and heart diseases (Wetli et al. 2004). These curative effects may be because of phytochemical agents inherent in them that have anti; inflammatory, cholesterol lowering, cancer preventing as well as stress reducing properties (Slimestad et al. 2007; Ye et al. 2013). It has also found application in the treatment of hepatitis (Akash et al. 2014) fever, headache, cholera, dysentery, and arthritis (Gomaa 2017; Gorinstain et al. 2010; Gupta et al. 2021). Onions has also been used as antifungal agent (Griffiths et al. 2002).

The organo-sulphur compounds derived from allicin constitute the effective agent that acts against bacteria (Tsao and Yin 2001; Saulis et al. 2002; Pszczola 2002; Shafiq et al. 2017; Teshika et al. 2019). Other health benefits derived from onions include enhancement of kidney function (Fredotović et al. 2021), wounds healing, pain relief and in the treatment of worms (Shri and Bora 2008). Thus, this wok intends to comparatively analyze the antimicrobial activities of different *Allium cepa* (onions) extracts on some bacterial and fungal *species*.

2. MATERIALS AND METHODS

2.1. Collection of Plant Material

The onion bulbs (*Allium cepa*) were purchased from a Market in Imo State Nigeria. The analysis was done at Nnamdi Azikiwe University Awka using the method of WHO (2003).

2.2. Collection of Microorganisms

These already identified microorganisms: *Staphylococcus aureus, Escherichia coli, Staphylococcus epidermidis, Pseudomonas aeruginosa Salmonella typhimurium*, *Klebsiella pneumoniae*, together with *Candida albicans,* were obtained from Bacteriology and Mycology sections of Clinical Microbiology laboratory of Federal Medical Centre (FMC), Owerri. The microorganisms were re-identified using biochemical tests (Cheesbrough 2006).

2.3. Onion Extraction using Cold water

The onion bulbs were dried in a hot air-oven under the temperature of 40˚C. It was reduced to powder using electronic blender, and 10g of the powdered sample was dissolved in 100ml of distilled water, sterilized at 121˚C for 15 min and allowed to stand for 2 days. It was then filtered, concentrated, and kept at 4ºC for further study (Hander 2005).

2.4. Chemical Extraction

The oven dried powdered onion sample was subjected to extraction using both ethanol and petroleum ether solvents at a solute – solvent ratio of 1:10 for 6 hours with the aid of a Soxhlet extractor according to Hander (2005). The extracted samples were preserved at 4ºC for further analysis.

2.5. Sterility Proofing of The Extracts

The sterility of the extracts was determined by culture using 2ml of the extract in 10ml of Muller Hinton broth and incubation at 37˚C for 24 hours. The absence of turbidity after incubation indicates sterility of the extracts.

2.6. Standardization of Inoculum

The standardization of inoculum to $1.5x10^8$ cfu/mL concentration was achieved by using (0.5 McFarland standard) Jorgensen and Turnidge (2015). The suspension was later diluted 1:100 with sterile broth, to obtain a cell number of approximately 10^6 cfu/mL.

2.7. Extract Dilution and Calculation of Concentrations

A 100mg/mL of the onion suspension was made by dissolution of 1g of onion extract in 10mL of sterile distilled water in a volumetric flask. This 100mg/mL was used as the stock solution. Thereafter, lower concentrations of 50, 25, 12.5, 6.25 and 3.125mg/mL were made from the stock. It is noted that 1g/10mL = 0.1g/mL = 100mg/mL. This was also done on the ethanol and Petroleum ether extracts.

2.8. Agar Well Diffusion Method

The method of Kirby Bauer (Shoeb 2008) was adopted to determine the antimicrobial potency of the onions extracts. The commercially produced antibiotic discs used as positive reference standards were Amykacin for the Gram-negative bacteria, Ceftriaxone for the gram-positive bacteria. Nistatin was used for the *Candida albicans* while distilled water was used as the blind control.

2.9. Minimum Inhibitory Concentration

The highest dilution of the extract that achieved inhibition (MIC) was determined by broth dilution method using Mueller Hinton broth as recommended by the Clinical Laboratory Standards Institute, CLSI (Jorgensen and Turnidge 2015).

2.10. Minimum Bactericidal Concentration

Here the tubes that showed no growth or least turbidity during the MIC were carefully selected out for sub-culturing according to Jorgensen and Turnidge (2015).

2.11. Minimum Fungicidal Concentrations

Here the tube that showed no growth or least turbidity during the MIC was carefully selected out for sub-culturing according to Jorgensen and Turnidge (2015).

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2.12. Statistical Analysis

Data obtained were subjected to ANOVA using SPSS ® Statistician Software (IBM 2011) and separation of differences between treatment means were carried out using DNMR test.

3. RESULTS

The result of the inhibitory effects of the onion hot water extracts against the test organisms was presented in Table 1. From the results, the onion extracts inhibited the growth of *Staphylococcus aureus* at 50mg/mL, 25 and 12.5mg/mL but partial inhibition was recorded at 6.25 and 3.125mg/mL concentrations. The higher the mg/mL concentrations of the onion extracts, the more the zone of inhibition. The same is true for other organisms.

Table 1: Effects of *Allium cepa* hot water extract on some bacteria and *Candida albicans*

Concentrations mg/mL	*Staph. aureus*	*Staph. Epidermidis*	*E. coli*	*Salmonella typhimurium*	*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*	*Candida albicans*
50.0	9.43	12.30	10.27	11.43	9.17	11.15	9.66
25.0	8.33	11.10	9.17	10.45	8.47	10.33	8.83
12.5	7.80	10.40	8.19	9.50	6.80	9.15	7.30
6.25	6.44	9.40	7.37	8.56	6.17	8.30	6.30
3.125	6.20	8.27	6,57	6.98	6.01	6.85	6,05

Key: Mean zones of inhibition greater than (>) 6.0=Sensitive. Mean zones of inhibition less than (<) 6.1=Resistant

The effect of *Allium cepa* cold water extract on selected bacteria and Candida species (mm) is presented in Table 2. Generally, cold water extract of *Allium cepa* inhibited the growth of all the microorganisms tested effectively from 12.5mg/mL concentration using 6.0 inhibition zone as standard. However, the inhibition zone is still high for *Staph. Epidermidis, Salmonella typhimurium and Pseudomonas aeruginosa* at 6.25 and 3.12mg/mL concentrations.

Table 2: Effects of *Allium cepa* cold water extract on selected bacteria and Candida species (mm)

Concentrations mg/mL	*Staph. Aureus*	*Staph. Epidermidis*	*E. coli*	*Salmonella typhimurium*	*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*	*Candida albicans*
50.0	9.24	12,22	10.17	11.23	9.13	11.10	9.53
25.0	8.33	11.10	9.07	10.43	8.49	10.03	8.63
12.5	7.80	10.39	8.10	9.40	6.78	9.10	7.20
6.25	6.40	9.35	7.27	8.47	6.15	8.20	6.09
3.125	6.21	8.26	6.47	6.96	6.00	6.87	6.01

Key: Mean zones of inhibition greater than (>) 6.0=Sensitive. Mean zones of inhibition less than (<) 6.1=Resistant

The effect of *Allium cepa* ethanolic extract on some bacteria and Candida albicans is presented in Table 3. The highest zones of inhibition were observed for *Staph. Epidermidis, Salmonella typhimurium, Pseudomonas aeruginosa* and *E. coli* respectively at 50mg/mL and 25mg/mL with decreased levels of inhibitions for others.

Table 3: Effects of *Allium cepa* ethanolic extract on some bacteria and *Candida albicans*

Concentrations mg/mL	*Staph. Aureus*	*Staph. epidermidis*	*E. coli*	*Salmonella typhimurium*	*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*	*Candida albicans*
50.0	9.43	12.50	11.10	11.93	9.96	11.55	9.86
25.0	8.23	11.53	10.17	10.75	8.76	10.63	8.80
12.5	7.70	10.77	9.53	9.80	6.90	9.55	7.40
6.25	6.60	9.70	8.53	8.76	6.25	8.60	6.60
3.125	6.33	8.57	7.53	6.98	6.15	6.25	6.01

Key: Mean zones of inhibition greater than (>) 6.0=Sensitive. Mean zones of inhibition less than (<) 6.1=Resistant

Table 4 presents the effect of *Allium cepa* petroleum ether extract on some bacteria and Candida albicans. The zones of inhibition were highest for *Staph. Epidermidis, Salmonella typhimurium, Pseudomonas aeruginosa* and *E. coli at* 50mg/mL and 25mg/mL with decreased levels of inhibitions for others.

The minimum inhibitory concentration of the various onion extracts on the different test organisms in mg/mL is presented in Table 5. *Staph. epidermidis, S. typhimurium* and *P. aeruginosa* were most inhibited by the highest dilution of the various extracts followed by *E. coli, Staph. aureus and Candida albicans* while *Klebsiella pneumoniae* only responded to the 25mg/mL concentration.

The minimum bactericidal and fungicidal concentrations of the various onions extracts is presented in Table 6. All the extracts were able to exhibit bactericidal effect on *S. typhimurium* at 3.125mg/mL concentration and *P. aeruginosa* at 6.25mg/mL respectively**.** Other organisms showed varying susceptibilities to 25mg/mL and 50mg/mL concentrations. However, there was no bactericidal effect of all the various extracts concentrations on *Staph. aureus* while *Candida albicans* responded to 50mg/mL fungicidal concentrations of all the extracts except cold water.

Table 4: Effects of *Allium cepa* petroleum ether extract on some bacteria and *Candida albicans*

Concentrations Mg/mL	*Staph. Aureus*	*Staph. epidermidis*	*E. coli*	*Salmonella typhimurium*	*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*	*Candida albicans*
50.0	9.23	12.53	11.30	11.97	9.92	11.75	9.81
25.0	8.33	11.52	10.27	10.95	8.66	10.53	8.89
12.5	7.50	10.75	9.55	9.70	6.84	9.45	7.39
6.25	6.48	9.70	8.43	8.86	6.33	8.50	6.65
3.125	6.22	8.50	7.03	6.88	6.32	6.35	6.00

Key: Mean zones of inhibition greater than (>) 6.0=Sensitive. Mean zones of inhibition less than (<) 6.1=Resistant

Table 5: Minimum Inhibitory Concentration of the onion extracts on the different test organisms in mg/mL

Test Organisms	Cold water extract (mg/mL)	Hot water extract (mg/mL)	Ethanolic extract (mg/mL)	Petroleum ether extract (mg/mL)
Staphylococcus aureus	12.5	12.5	12.5	12.5
Staphylococcus epidermidis	3.125	3.125	3.125	3.125
Escherichia coli	6.25	6.25	3.125	3.125
Salmonella typhimurium	3.125	3.125	3.125	3.125
Klebsiella pneumoniae	25.0	25.0	25.0	25.0
Pseudomonas aeruginosa	3.125	3.125	3.125	3.125
Candida albicans	12.5	12.5	12.50	12.5

Table 6: Minimum bactericidal and fungicidal concentrations of the onion's extracts

Test Organisms	Cold water extract (mg/mL)	Hot water extract (mg/mL)	Ethanolic extract (mg/mL)	Petroleum ether extract (mg/mL)
Staphylococcus aureus	NA	NA	NA	NA
Staphylococcus epidermidis	50	50	25	25
Escherichia coli	50	50	25	25
Salmonella typhimurium	12.5	12.5	12.5	12.5
Klebsiella pneumoniae	NA	NA	50	50
Pseudomonas aeruginosa	6.25	6.25	6.25	6.25
Candida albicans	NA	50	50	50

Key: NA = No activity

4. DISCUSSION

The findings on the response of *Staphylococcus aureus* therefore support the work of Azu et al. (2007) and Benkeblia (2004), who reported that *Staph. aureus* was sensitive to onion extracts but at higher concentrations. From the results in Table 2 to 4, the hot water and ethanolic extracts were more effective against *Staph. aureus* than the cold water and petroleum ether extracts.

The effect of the onion extracts on the normal flora of the eye, *Staph. epidermidis* as shown in Tables 2 to 5, revealed that *Staphylococcus epidermidis* was highly susceptible to the onion extracts than the *Staphylococcus aureus*. The results therefore support the work of Azu et al. (2007) and Fredotović et al. (2021), that onion extract was effective against *Staphylococcus species*. The findings of antibacterial activities of the onion extracts against *E. coli* (Tables 2 to 5) showed that the cold water, hot water, ethanolic and petroleum ether extracts of the onion were effective against the test organism, *E. coli*. The results of the research therefore have been in agreement with the work of Benkeblia (2004), who said that *E. coli* are sensitive to onion extract. Tables 4 to Table 6, also showed that *S. typhimurium* was sensitive to the cold water, hot water, ethanolic and petroleum ether extracts of the sample (Benkeblia 2004; Fredotović et al. 2021). From the results in Tables 2 to 5, *Allium cepa* extracts could therefore be considered as natural preservative or food additive and also serve as additional method of controlling food borne pathogens when added to food salads. The results of the present study, therefore, have agreed with the findings of Zohri et al. (1995) and Induja and Geetha (2018) who pointed out that onion extract inhibits the *in-vitro* growth of *E. coli* and *S. typhimurium.*

From the results in Table 2 to 5, the onion extracts were found to be considerably less effective against the gram negative *Klebsiella pneumoniae.* This could be due to the presence of capsule in *Klebsiella pneumoniae.* The results therefore have been in agreement with Griffiths et al. (2002) who reported that onions oil and extracts were almost ineffective against some Gram-negative bacteria.

The results in Table 2 to 5 showed that the onion extracts were effective against *Pseudomonas aeruginosa*. This could be due to the oil components of the extracts which suffocate the obligate aerobe, *Pseudomonas aeruginosa.*

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The results, therefore, support the ethnomedicinal claim that onion extract is being used to prevent bacterial wound infections (Zohri et al. 1995).

Table 2 to 5 also showed the antifungal activities of *Allium cepa* extracts against *Candida albicans*. The result showed that onion extracts were effective against *Candida albicans* but at a higher concentration. It is therefore, in agreement with Ross (2001) and Induja and Geetha (2018) findings that; Onion extract was effective against *Candida albicans* and other *Candida species,* though the inhibition was dose – dependent.

The minimum inhibitory concentration (MIC) of the onion extracts on test organisms is shown in Table 6. The results showed that the inhibitory effects of the onion extracts differ from one microorganism to another. From the results, all the onions extracts have MIC of 3.125mg/mL on *Staphylococcus epidermidis.* The cold and hot water extracts have MIC of 6.25mg/mL on *Escherichia coli* while the ethanolic and petroleum ether extracts had MIC of 3.125mg/mL on the same organism. The MIC of the extracts on *Pseudomonas aeruginosa* and *Salmonella typhimurium* was 3.125mg/mL. The MIC of the extracts on *Staphylococcus aureus* and *Candida albicans* were 12.5mg/mL. The MIC of the extracts on the capsulated *Klebsiella pneumoniae* was 25mg/mL. The results of the MBC and MFC (Table 6) supported the work of previous researchers (Lai and Roy 2004; Santas et al. 2010).

From the statistical analysis of the Bioassay, 5% degree of freedom, there was a significant difference between the zones of inhibition by cold water extract and hot water extract on the test organisms, likewise this correlates with the solvent extracts. But there was no significant difference between the effects of aqueous and solvent extracts.

5. Conclusion

This study maintained that onion extracts are effective against some gram-positive bacteria, gram negative bacteria and *Candida albicans. C*old water extract performed better than hot water extract in inhibiting most of the experimental organisms. Again, solvent extract was better than aqueous extracts. Extracts of onions could be used as herbal natural medicine, and antimicrobial agent.

Authors contribution

Okonkwo IF conceived, designed, supervised and edited the work. Achilike KM conducted the experiment, analyzed the data and wrote the manuscript.

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