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In vitro assessment of antibacterial activity of crude extracts of onion (*Allium cepa* L.) and shallot (*Allium aescalonicum* L.) on isolates of *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923), and *Salmonella typhi* (ATCC 19430)

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Abstract
Antibacterial activity of crude extracts of two *Allium* species namely *Allium cepa* Linn (onion) and *Allium aescalonicum* Linn (shallot) cultivated in Ghana was investigated on isolates of *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923), and *Salmonella typhi* (ATCC 19430) by the agar well diffusion method. Broth cultures of the test bacterial isolates were standardised to 0.5 McFarland (1.0×10^6 cfu/ml) and seeded on nutrient agar. Crude extracts of the onions and shallots were prepared from samples bought from the market. 75µl of undiluted and diluted crude extracts were introduced into corresponding agar wells and incubated at room temperature (approximately 37 °C) for susceptibility test. 15µg/mL of Ciprofloxacin was set as control. After 24 hours of incubation, zones of inhibition were measured and recorded in millimetres. The undiluted extracts exhibited greater inhibitory activity on the test organisms than the diluted extracts. *Salmonella typhi* was observed to be susceptible to the crude extracts though resistant to ciprofloxacin. Diluting crude extracts of both onion and shallot beyond 1:2 dilution had no antibacterial action on the isolates. Further research is recommended on the pharmacokinetics of the active components in the crude extracts. Eating raw onions and shallots should be encouraged.

Keywords: *Allium* sp., Crude extract, bacterial isolates, zone of inhibition.

1. Introduction
Onions are bulb vegetables used as flavouring and seasoning agents in many types of cuisines. They form an essential part of the daily diet, consequently there is a year round demand for it. They are either added whole and glazed for stews or chopped or minced for soup and sauces. Some would rather chop or slice raw onion and use as garnish for salads. In Ghana there is a preference for red and yellow onions identified as *Allium cepa* Lnn at the Department of Botany, University of Ghana. Reports from previous research (Hamza, 2015) [6] show onions as effective against common cold, heart diseases, diabetes, osteoporosis, coughs and sore throat. Onions are rich in proteins, carbohydrates, sodium, potassium and phosphorous (Lampe, 1999) [8]. Both red and yellow *A. cepa* are cultivated extensively in the northern parts of Ghana under relatively dry environmental conditions with organic or inorganic fertilizers. Onion bulbs are found to contain a good number of phytochemicals, including the organosulfur compounds (Dorsch, 1996; Jeffery and Herbert, 2003) [3, 7]. The relative pungency of onion has been attributed to both genetic and environmental factors (Ghalia 2016) [5]. Flavour of onions is due to development of sulphur compounds which occurs throughout the growing period (Meriel *et al*., 2004: Newman *et al*., 2006) [9, 10]. Water supply plays a large role in determining how pungent and flavourful the onion will be. The hotter the conditions the more sulphur compounds will be produced, leading to a more pungent flavour (Newman *et al.,* 2006) [10]. These findings imply there is high variability in flavour strength depending on where, when and how the onion is grown.
Shallots (*Allium aescalonicum*), are also members of the onion family used as flavouring agents in the preparation of soups and sauces or for grilled fish. Shallots consist of a cluster of small to medium-sized bulbs with a reddish brown to orange brown outer skin. They are usually used in combination to other spice blends and condiments. Raw shallots are grounded with pepper and tomatoes and eaten with other foods. They are also fried in oil and added as toppings to stir-fried foods to give a crunchy texture or caramelized taste.

Shallots are cultivated extensively along the coast of Ghana in the Keta basin, overlooking the Atlantic sea on beds usually measuring approx. 2m x15m.

High levels of antioxidants activity have been reported for shallot due to high levels of phenolic compounds. Shallots just as onions works to inhibit enzymes involved in cholesterol and fatty acid synthesis *in vitro*. Other benefits include, improving the immune system, preventing cataracts and macular degeneration, preventing arthritis, improving circulation, and decreasing skin wrinkling (Fleischauer *et al.*, 2000) [4].

This research study is aimed at accessing the antibacterial activity of the crude extracts of *Allium cepa* and *A. aescalonicum* cultivated and sold in Ghana against selected food-borne pathogenic bacteria such as *Escherichia coli*, *Staphylococcus aureus* and *Salmonella typhi* and to determine the susceptibility patterns of the selected food-borne pathogens to the crude extracts.

2. Materials and Methods

Antibacterial activity of crude extracts of two *Allium* species (*A. cepa* and *A. aescalonicum*) was evaluated on three bacterial isolates ie *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923), and *Salmonella typhi* (ATCC 19430).

2.1 Collection, identification of plant materials and test microorganisms

The test microorganisms were obtained from the microbiology laboratory at the Centre for Plant Medicine Research (CPMR), Mampong-Akuapem, Ghana. The bacterial strains were sub-cultured on nutrient agar slants and kept at 4 ºC until ready for use.

The plant materials (shallots and onions) were obtained from the market and were transported to Department of Botany, University of Ghana, Legon, (Accra-Ghana) for identification.

2.2 Extraction of the plant materials

Fresh *A. cepa* and *A. aescalonicum* bulbs were washed with freshly prepared sterile distilled water. The outer covering of the bulbs were peeled off and the fleshy part of the onions and shallots rewashed separately with the distilled water. A part of 200g of each onion or shallot bulbs were cut into small parts and blended. The juice from the resulting pastes were then squeezed out using muslin cloths and filtered with Whatman No. 1 filter papers into labelled storage bottles and stored at 4 °C until use.

Two fold dilution concentration of each crude extract (1:0, 1:2, 1:4 and 1:8) were prepared using sterile distilled water and used immediately.

2.3 Preparation of inoculum and antibacterial activity determination

About 18 hour broth cultures of the test bacterial isolates were re-suspended into sterile nutrient broths and standardized according to the Clinical and Laboratory Standards Institute (CLSI) (2012), to 0.5 McFarland (1.0×10^6 cfu/ml).

Antibacterial activity of the crude extracts were determined by the agar-well diffusion method. Twenty-five microliter (25µl) of the standardized cell suspensions of each test organism was plated on Mueller-Hinton agar plates (5mm thick), labelled for identification and incubated overnight at 37ºC. Wells were then bored into each seeded agar plate using sterile 6mm diameter cork-borers. Agar plugs were removed and 75µl of each crude extract in 1:0, 1:2, 1:4 and 1:8 dilutions were introduced into their corresponding wells and incubated at 37 °C for 24 hours. 75µl of 15µg/ml of ciprofloxacin was used as control. The plates were observed for zones of inhibition after 24 hours of incubation. Measurements were taken and recorded in millimetres.

2.4 Statistical analysis

The mean inhibition zones for each extract were analysed with SPSS and differences between the extract types were compared to 15µg/ml ciprofloxacin at 0.05 significance level.

3. Results and Discussion

3.1 Antibacterial activity of crude extract of *Allium aescalonicum* (shallots) and 15µg/ml ciprofloxacin against bacterial isolates.

All the test microbes were susceptible to undiluted crude extract (1:0) of the *A. aescalonicum.* The highest zone of 23.0mm (mean) was recorded for *S. typhi* (ATCC19430). At dilution of 1:2, the zone of inhibition was reduced to a mean of 13.3mm. *S. typhi* was however resistant to ciprofloxacin (Table 1). *E. coli* and *S. aureus* were susceptible to both the crude extracts and to ciprofloxacin with varying zone of inhibitions. Susceptibility of *E. coli and S. aureus* to the undiluted crude extract of shallots and ciprofloxacin showed reliable difference between their activities i.e. $F(1, 8) = 2640.58$, $P<0.05$ and $F(1, 8) = 571.43$, $P<0.05$ respectively.

Table 1: Antibacterial activity profile of diluted and undiluted extract of *Allium aescalonicum* against bacterial strains.

	Mean inhibition ZONE (mm)				
Test Organism	**Undiluted**	**Dilution**			**15 ug/mL ciprofloxacin**
	1:0	1:2	1:4	1:8	
S. typhi (ATCC19430)	23.0	13.3	0.0	0.0	0.0
E. coli (ATCC25922)	20.3	16.7	0.0	0.0	27.0
S. aureus (ATCC25923)	12.0	0.0	0.0	0.0	16.0

3.2 Antibacterial activity of crude extract of *Allium cepa* (yellow onions) and 15 ug/mL ciprofloxacin against bacteria strains.

Antimicrobial activity was observed only for the undiluted extract of yellow onions. The different dilutions were not effective against the selected microbes (Table 2). A comparison of susceptibility of *E. coli and S. aureus* to undiluted crude extract of yellow onions and ciprofloxacin was $F(1, 8) = 14222.22$, $P<0.05$ and $F(1, 8) = 208.33$, $P<0.05$ respectively.

Table 2: Antibacterial activity profile of diluted and undiluted extract of *A. cepa* (yellow onions) against bacterial strains

	Mean inhibition Zone (mm)				
Test Organism	**Undiluted**	**Dilution**			**15 ug/mL ciprofloxacin**
	1:00	1:2	1:4	1:8	
S. typhi (ATCC19430)	19.3	0.0	0.0	0.0	0.0
E. coli (ATCC25922)	11.0	0.0	0.0	0.0	27.0
S. aureus (ATCC25923)	13.5	0.0	0.0	0.0	16.0

3.3 Antibacterial activity of extract of *Allium cepa* (red onions) and 15 ug/mL ciprofloxacin against bacteria strains.

Susceptibility to crude extracts of red onions was observed at 1:0 and 1:2 dilutions for *S. typhi* and *E. coli* (Table 3). *S. aureus* was resistant to 1:2 dilution of the crude extract. ANOVA at 95% confidence interval between undiluted crude extract and ciprofloxacin gave the following. $F(1, 8) = 204.55$, $P<0.05$ and $F(1, 8) = 346.15$, $P<0.05$ respectively.

Table 3: Antibacterial activity profile of diluted and undiluted extract of *A. cepa* (red onions) against test bacteria.

	Mean inhibition Zone (mm)				
Test Organism	**Undiluted**	**Dilution**			**15 ug/mL ciprofloxacin**
	1:00	1:2	1:4	1:8	
S. typhi (ATCC19430)	18.7	12.0	0.0	0.0	0.0
E. coli (ATCC25922)	28.5	12.0	0.0	0.0	27.0
S. aureus (ATCC25923)	13.0	0.0	0.0	0.0	16.0

Comparison of antibacterial activity profiles of undiluted extracts of *Allium* species and 15 ug/mL ciprofloxacin.

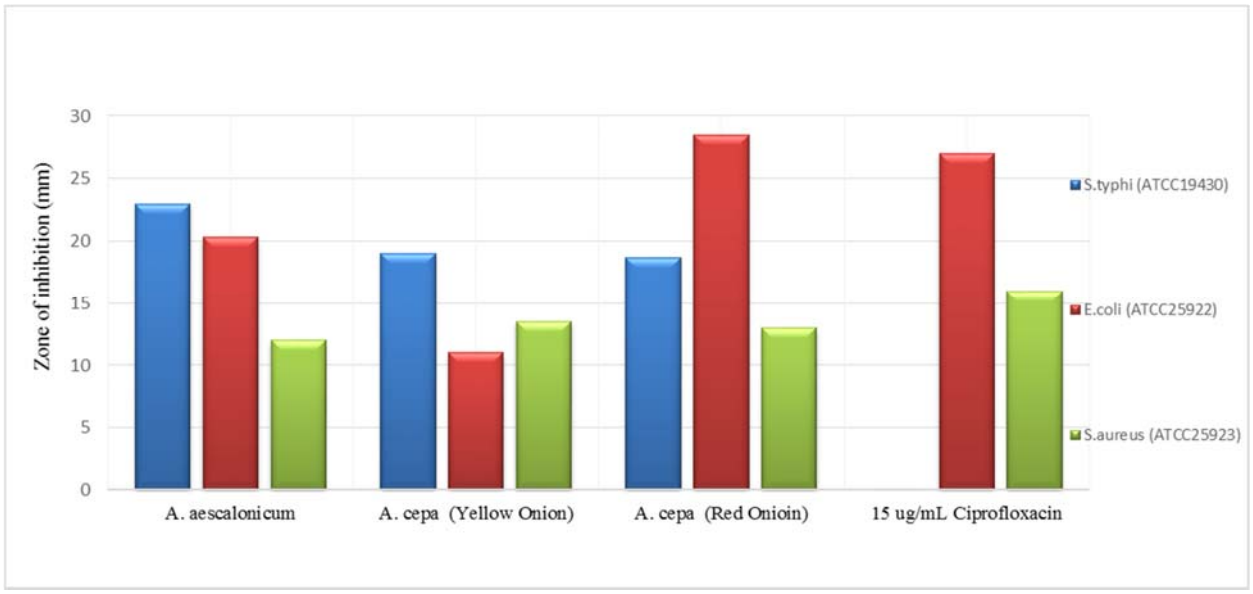

Fig 1: Antibacterial activity profile of undiluted extract of *Allium* sp and 15ug/mL ciprofloxacin against selected microbes.

Susceptibility of *S. typhi* (ATCC19430) to the undiluted extracts of the three species of *Allium* gave $F(2, 12) = 64.81$, $P<0.05$ (Figure 1) where *S. typhi* was more susceptible to *A. aescalonicum.* This was followed by yellow onion and finally red onions.

There was statistically reliable difference in susceptibility for *E. coli* (ATCC25922) against the undiluted extracts of the three species of *Allium* and Ciprofloxacin [$F(3, 16) = 9084.76$, $P<0.05$]. *A. cepa* (red onion) displayed the highest effectiveness and is followed by Ciprofloxacin. Shallots and yellow onion recorded the least effectiveness.

Effectiveness of crude extracts and ciprofloxacin against *S. aureus* (ATCC25923) gave the following $F(3, 16) = 251.81$, $P<0.05$. Indicating that Ciprofloxacin was more effective. This was followed by yellow onion and then red onion. Shallots exhibited the least effectiveness.

4. Discussion

The results of this study demonstrates antimicrobial activity of crude extracts of *Allium* species sampled against the test bacterial isolates (Tables 1-3). Red and yellow onions are considered as one of the best natural sources of quercetin, a bioflavonoid that is particularly well suited for scavenging free radicals. Aside from its antioxidant properties, quercetin has been found to possess anti-fungal, anti-bacterial, and anti-inflammatory properties. In addition to quercetin, allium plants produce allicin when the plant is crushed or chopped. Allicin has also been reported to possess antimicrobial properties. As a member of the *Alliaceae* shallots have also been reported to produce allicin when crushed or chopped. This could account for the antibacterial properties observed.

Generally, the zones of inhibition observed for the diluted extracts of onions and shallot were smaller indicating

reduction in antimicrobial activity. Susceptibility of *S. typhi* (ATCC19430) to crude extract of *Allium sp* implies an infection of this isolate can be treated with crude extracts of onion and shallots. Similarly, crude extracts of red onions can be used for *E. coli* (ATCC25922). Generally, red onions showed greater antimicrobial activity than yellow onions. They also had more pungency than the yellow. Zones of inhibitions observed for *S. aureus* (ATCC25923) treated with the crude extracts were relatively low, implying resistance compared to ciprofloxacin. Diluted extracts of yellow onions showed no antimicrobial activity.

5. Conclusion

Crude extracts of both red and yellow onions as well as shallots demonstrated antimicrobial activity against the test bacterial isolates and is comparable to the action of ciprofloxacin. Dilution of crude extracts results in reduction of antimicrobial activity.

6. Recommendation

Further research is recommended on the pharmacokinetics of the active components in crude extracts. Eating raw onions and shallots should be encouraged.

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