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Antibacterial Activity and Anti-nutrient Composition of White and RED *Allum cepa* (Onion)

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The research was carried out to determine the anti-nutrient values and antimicrobial activity of Red and White *Allium cepa*. Two different solvents (ethanol and methanol) were used for the extraction of the samples. The extracts showed the presence of taninns, saponins, alkaloids and steroids but terpenoids and anthraquinones were absent in both extracts. The anti-nutrient values obtained showed that white onions has higher flavonoid (20.88 ± 0.24 %) than the red onion (19.60 ± 0.19 %) and tannins recorded the lowest value (0.24 ± 0.001 and 0.22 ± 0.003) for both white and red onions respectively. The extracts were screened for their antibacterial activity using the agar-well diffusion method. They were tested against Gram-positive bacterium (*Staphylococcus aureus*) and Gram-negative bacterium (*Pseudomonas aeruginosa*). The ethanol extract of the white onion showed highest activity against the test organisms with a highest zone of inhibition of 34.79 mm on *Staphylococcus aureus*, while the white onion methanol extract, recorded the lowest (12.09 mm) for *Pseudomonas aeruginosa* at 50 mg/ml. The minimum inhibitory concentration of the potent onion extracts showed positive inhibitory effect when an undiluted extracts was used, however, growth was observed at the lowest concentration (6.25 mm and 12.5 mm) of white and Red onion extracts. From these results, it be concluded that the white onion extracts showed more efficacy on the test organisms than the red onion extracts and also the ethanolic extracts are more active than the methanolic extracts. The efficacy of this plants extracts on the tested organisms may be attributed to the phytochemicals present on the extracts.

Keywords: Onions, extracts, agar-well diffusion, inhibition, anti-nutritient

INTRODUCTION

Endowed with numerous useful medicinal plants, Nigeria is yet to take full advantage of her abundant resources in attaining a high level of self sufficiency in drug production. Plants have always been a component of man kind's healthcare system which can be directly or indirectly (Agarwal *et al*; 2007). The use of herbal medicine in Africa has greatly elevated and enhanced the primary health care system (Radcliffe-Smith, 1987). Plants are widely used in many indigenous systems of medicine for therapeutic purposes and are increasingly becoming popular in modern society as alternatives to synthetic medicine (Chikezie *et al*., 2015) and about 70-80% of Asian and African populations rely on this for their primary healthcare needs (Chikezie *et al*., 2015; Neergheen-Bhujun, 2013). Herbs can be defined as plants which lack permanent woody stem that produce seeds and flowers and die after a particular season. They are medicinal in nature and can also be used for horticultural purposes. However, in traditional herbal system, an herb is a small, non-woody plant, treasured for its medicinal savory or aromatic functions. Herbal medicine therefore is a natural remedy derived from herbs (Neuwinger, 2000). Herbal medicine is generally cheaper, accessible or readily available and more culturally acceptable to many because of the believe that they cause less side effects than some synthetic drugs (Carlson, 2002; Dey and De, 2015). According to Okoli *et al*. (2007), traditional medicinal practices in Africa on the date as far back as 4000 years and were the sole system for healthcare before the advent of orthodox or modern medicine. Beyond Africa (Nigeria), the world is experiencing an increasing rate of resistance by pathogens to some of the synthetic drugs as well as the struggle against cancer and AIDS which have not found treatments from modern medicine. Consequently, this has challenged the scientific community to initiate research programme aimed at seeking solutions from plant species (Msuya, 1998). Therefore, the importance

of herbal medicines in the life of Africans cannot be over-emphasized. Many spices such as onions and garlic have been considered medicinal plants which can be potentially used for controlling food borne pathogens in place of chemicals and antibiotics (Serthi *et al*., 2013; Purseglove, 2005; Abdou *et al*., 2001). *Allium* is the largest and important representative genius of the Liliaceae, the family *Alliaceae,* comprises of about 450 species (Augusti, 1996). Onion is a swollen edible bulbous plant used as vegetable, having a pungent taste and smell and composed of several layers (Rui, 2004). They are of different shape and size. They are of various types which include the three common ones, namely: brown, red and white onions. The brown onion has a brown or yellow skin and a creamy flesh, with a strong flavor. The red onions have a purple red skin, and are abundant in polyphenols, flavonoids, alkaloids, tannin, thallonal (Abdelsalam *et al*., 2014); while the white onion has white skin and possess the strongest flavor after brown (Sagdic and Ozcan, 2003). In Nigeria, onions have various local names, Albasa in Hausa; Alubosa in Yoruba while Igbo call it yabasi (Aiyeloja and Bello, 2006). Onions are believed to have evolved from the arid region of western Asia, Africa and some part of Europe with different climatic condition. Onion is composed mainly of water (up to 90%) with a negligible nutrient content (Ravindran, 2017). It contains vitamins A and C, while the white part of it has calcium. It is also rich in sulfur, an essential element that kills or inhibits fungus infection (Kumar *et al*., 2010). Onions are considered as one of the small number of vegetables which reduce heart disease risk. This beneficial effect is attributed to its vitamin B6, which lowers homocysteine levels, an important risk factor for heart attacks and strokes (Okoli *et al*., 2007).Onions have been described to possess many medicinal properties (Megan, 2015; Marsun, 2015; Kumar *et al.,* 2010. The reported medicinal properties of onions have led to this research to evaluate the anti-nutritient and antimicrobial potential of *Allium cepa* (Onion).

MATERIALS AND METHODS

Fresh bulbs of *Allium cepa* (onions) were purchased from Ungwan Rimi market, Kaduna north, Kaduna State, Nigeria. The onions were taxonomically identified and authenticated in the Department of Biological Sciences, Faculty of Science, Kaduna State University, Kaduna, Nigeria.

Collection and maintenance of test organisms

The test organisms were all of human pathogenic organisms from clinical isolates. One Gram positive and One gram negative bacteria were selected and the isolates are *Staphylococcus aureus and Pseudomonas aeruginosa.* The organisms were obtained from the department of Microbiology; Kaduna state University (KASU).The organisms were collected on sterile agar slants and were kept as stock culture in the refrigerator at 4°C

Extraction of bioactive constituent

The onions that were obtained were cleaned and washed with sterile distilled water. Using disinfected knife and chopping board, the samples were chopped into smaller pieces, crushed in a mortar using a pestle and liquefied in blender. The extract was then sieved using muslin/cheese cloth into a sterile conical flask. The filtrate was then evaporated at 45°C to dryness and the dried substance was then kept in sterile bottle under refrigerated condition for further analysis. This was considered as 100% raw extracts (Yassir *et al*., 2010)**.**

Preparation of ethanolic and methanolic extracts

Ten grams of each dried powder sample was extracted with 100 ml of 80% ethanol and methanol in a screw capped flask and was shaken and kept at room temperature for 24 h. The extract was then filtered through whatmann paper (no1) at 40°C**.** Then the concentrated extract was then stored in a freezer at 10°C for further analysis. Stock solution of the extract was prepared by diluting the dried extract with dimethyl sulfoxide (DMSO) solution (Aradhana *et al*., 2013).

Determination of phytochemical constituents of the extracts

Chemical analysis of the dried extracts was carried out for the qualitative determination of phytochemical constituents as described by Akinyemi *et al.* (2005); Junaid *et al.* (2006) and Prashant *et al.* (2011).

Determination of anti-nutritional factors in the samples

Determination of tannins

The tannin content was estimated spectrophotometrically by Folin-Denis method as described by Pearson, (1976). The samples were dried at 55±1ºC and were ground to pass through a sieve of 1 mm diameter. Tannins extraction was done by weighing 400 mg ground sample into conical flask, and 40 ml diethyl ether containing 1% acetic acid (v/v) was added and mixed to remove the pigment material. The supernatant was carefully discarded

after 5 min and 70% aqueous acetone was added and sealed with cotton plug and covered with aluminium foil and was stirred in an electric shaker for 2 h for extraction. It was then filtered through Whatman filter paper No.1 and the sample was kept in the refrigerator at 4ºC for further analysis.

Standard calibration curve preparation

From the stock solution of tannic acid (0.5 mg/ml), 0, 10, 20, 30, 40 and 50 µl were transferred into test-tubes and the volumes were made up to 1.0 ml. These contain tannic concentration of 0, 5, 10, 15, 20 and 25 µg respectively. Then 0.5 ml of Folin reagent and 2.5 ml 20% sodium carbonate were added and the whole content was mixed properly and left 40 min. Absorbance of each concentration was taken at 725 nm using spectrophotometer to prepare a standard curve. The tannin content of the onion samples was then estimated according to the procedure of Makkar *et al.* (1993). About 50 µl of tannins extract for each sample were taken into test tubes and the volumes were made up to 1.0 ml with distilled water. Then 0.5 ml of Folin-Ciocalteu reagent was added and mixed thoroughly. Then 2.5 ml of 20% sodium carbonate solution was added, mixed and kept for 40 minutes at room temperature. Optical density was taken at 725 nm in the spectrophotometer and the concentration was estimated from the standard curve.

Calculation

$$\% \text{ tannin (mg/100g)} = \frac{A_n \times C \times D_f}{A_s \times W \times 100}$$

Where:

A_n = absorbance of test sample
A_s = absorbance of standard tannic acid
C = concentration of standard tannic acid (mg/ml)
D_{fb} = dilution factor = V_{ex}/V_a
W = weight of test sample (mg)
V_{ex} = total volume of extract
V_a= volume of extract analyzed

Determination of saponin

A gravimetric method of AOAC (1990) was employed using soxhlet extractor and two different organic solvents were used for the extraction. Two grams of the sample was weighed into a thimble and put in a soxhlet extractor with a condenser fitted on top. Extraction was carried out with acetone in a 250 ml round bottom flask for 3 hours, after which another weighed 250ml round bottom flask containing methanol was fitted to the same extractor and extraction was allowed to continue for another 3 h. At the end of the second extraction, the methanol was recovered by distillation and the flask was oven-dried to remove the remaining solvent in the flask. The flask was then allowed to cool in a desiccator and then weighed.

Calculation

$$\% \text{ Saponin} = \frac{A - B \times 100}{W}$$

Where

A = weight of flask and extract (saponin)
W = weight of sample
B = weight of empty flask

Determination of alkaloid

The gravimetric method of Harbone, (1998) was adopted. 5 g of the sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added to the sample and was allowed to stand for 4 h. The extract was filtered and concentrated on water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the preparation is completed. The whole solution was allowed to settled, and the precipitate was filtered in a weighed filter paper and was washed with 1% ammonium hydroxide solution. The precipitate in the filter paper was dried in the oven at 60ºC for 30 min and was reweighed.

Calculation

$$\text{Percentage of total alkaloids (\%)} = \frac{\text{Weight of residue} \times 100}{\text{Weight of sample taken.}}$$

Determination of cyanogenic glycoside (HCN)

The determination of cyanogenic glycoside was carried out using alkaline picrate method of Onwuka, (2005). Five gram of sample was weighed and dissolved in 50 ml of distilled water in a corked conical flask. The cyanide extraction was allowed to stay overnight and filtered. Different standard concentrations of KCN solution containing 0.1 to 1.0 mg/ml cyanide were prepared.To 1 ml of the sample filtrate and standard cyanide solution in test tubes, 4ml of alkaline picrate solution will be added and incubated in water bath for 15 min. After colour development, the absorbance will then be taken at 490 nm against a blank containing only 1ml distilled water and 4 ml alkaline picrate solution. The cyanide content will be extrapolated from the cyanide standard curve.

Calculation

$$\text{Cyanogenic glycoside (mg/100g)} = \frac{\text{C (mg)} \times 100}{\text{Wt. of sample}}$$

Where: C (mg/100g) = concentration of cyanide content read off the graph.

Determination of flavonoids

The method of Boham and Kocipai-Abyazan (1974) was adopted. Ten grams of the samples was extracted thrice with 100ml each of 80% aqueous methanol at room temperature. The whole solution was filtered through whatman filter paper No. 42. The filtrate was transferred into a crucible and evaporated to dryness over water bath and weighed to constant weight.

Calculation

$$\% \text{ Flavonoid} = \frac{\text{Weight of Flavonoid} \times 100}{\text{Weight of sample}}$$

Test for antimicrobial activity

The antimicrobial screening of the ethanolic and methanolic extract was done as described by Shinkafi and Dauda, (2013). Nutrient agar was poured in sterile Petri dishes and was allowed to solidify. 1.0 ml of the test culture was dropped on the agar and the organism was spread all over the surface of the agar using a spreader. Wells of approximately 5 mm in diameter were made on the surface of the agar medium using a sterile cork borer. The plates were turned upside down and the wells labelled with a marker. Each well was filled with 0.2 ml of the extract. Ciprofloxacin and distilled water were used in the nutrient agar plates as positive and negative controls. The plates were incubated aerobically at 37^{o}C for 24 h. Sensitivity of the organisms to the extract (zone of inhibition) was recorded.

Minimum inhibitory concentration (MIC) of the extracts

The minimum inhibitory concentration (MIC) is the concentration at which antibacterial agents (plants extracts) experiences the complete inhibition of the microorganism growth (Aradhana *et al*., 2013) after overnight incubation period. Into four sterile test tubes was dispensed 2 ml of distilled water. To the first test tube, 2 ml of 50% extracts was added and serially diluted into the rest of the three test tubes, followed by adding 2 ml of prepared Mueller Hington broth (MHB) to each of tube. Each of the tube containing MHB and the extracts were then inoculated with a wire loop full of the standardized organism. The tubes were incubated at 37^{o}C for 24 h. The concentration of the least clear tube after incubation that shows no visible turbidity was recorded as the MIC (NCCLS, 2002).

RESULTS

The results of phytochemical screening on the different onion extracts are shown in (Table 1). The following phytochemicals were found to be present in the extracts: *taninns, saponins*, alkaloids steroids and all the extracts did not show the presence of *terpenoids and anthraquinones*. Table 2 showed that the onion samples are rich in flavanoids and alkaloids. The onion extracts inhibited the isolates when compared to the standard antibiotics used (Tables 3-5). The white methanolic extract showed higher activity against *Staphylococcus aureus* even at the lowest concentration of 62.5 mg/ml than the red onion extract.

DISCUSSION

The results obtained from this research, revealed the antimicrobial and phytochemical properties of red and white onion ethanolic and methanolic extracts. The results showed that the test bacteria was inhibited by various concentration of the extracts, though the higher the concentration the higher the zone of inhibition. It was discovered that the ethanolic extract of white onion exhibit the highest antimicrobial effect on all the tested organisms with the highest zone of inhibition of 34.79 mm at 50.0 mg/ml concentration. The result clearly showed that the white onion extracts has more inhibitory effect than its red counterpart. However from the result obtained, *Staphylococcus aureus* showed more sensitivity to both red and white onion extracts than the *Pseudomonas aeruginosa.* The demonstration of activity against the test bacteria provides scientific bases for the local usage of these plants in the treatment of various ailments (Ankri and Mirelman, 1999). The fact that these extracts were active against both Gram- positive bacterium and Gram -negative bacterium tested may indicate a broad spectrum of activity (Prescort, 2005). The Gram-positive bacterium was more sensitive to the extract because Gram-positive bacteria are normal floral that when altered may be pathogenic, while Gram-negative bacterium is less sensitive to the extracts. However, the ethanolic extract of white onion possesses higher antimicrobial effect on the test organisms than the methanolic extract, which suggests that the solvent used during the extraction also plays a role in the activity of the extracts. The effectiveness of ethanol can be due to its polarity than methanol which may results in extracting

Table 1. The results of phytochemical screening for both the white and red onions.

	White Onions		Red Onions	
Phytochemicals	**Ethanolic**	**Methanolic**	**Ethanolic**	**Methanolic**
Flavonoids	+	+	+	+
Tannins	+	+	+	+
Alkaloids	+	+	+	+
Saponins	+	+	+	+
Steroids	+	+	+	+
Terpenoids	_	_	_	_
Cardiac Glycosides	+	+	+	+
Phenols	+	+	+	+
Reducing sugar	+	+	+	+
Anthraquinones	_	_	_	_

KEY: + Present and - Absent

Table 2. Antinutritient composition of the onionsamples

Antinutritient	**Percentage (%)**	
	White Onion	**Red Onion**
Flavonoid	20.88 ± 0.24	19.60 ± 0.19
Alkaloids	6.83± 0.07	4.82 ± 0.02
Tannins	0.24± 0.01	0.22 ± 0.03
Cyanogenic Glycoside	7.63± 0.05	9.09 ± 0.06

Results are means of Triplicate Determination ± Standard deviation (SD).

Table 3. Zone of inhibition of Methanolic extracts of red and white *Allium cepa* at different concentration on different isolates

Concentration (mg/ml)	***Pseudomonas aerogenosa* (mm)**		***Staphylococcus aureus* (mm)**	
	Red Onion	**White Onion**	**Red Onion**	**White Onion**
50.0	19.75 ±0.021[a]	12.09±0.021[a]	19.23±0.021[d]	17.35±0.022[d]
25.0.	12.98 ±0.014[b]	7.13±0.021[b]	12.15±0.021	13.24±0.021
12.5	6.16 ±0.156[c]	4.73±0.07[c]	7.25±0.07	8.21±0.014
6.25	0.00±0.00	0.00±0.00	3.81±0.014	4.15±0.07[d]
Control	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Ciproflaxacin	21.93±0.07	20.23±0.05	22.23±0.07	20.69± 0.08

Values are means ± standard deviation, (n =3). Values followed by different letters are significantly differently ($P \leq 0.05$) from each other for each isolates between the different extract concentration. n = number of replicates

more active ingredients. The presence of some phytochemicals in the onions is a function of the antibacterial activity of onion extracts against the pathogens as they play important roles in bioactivity in medicinal plants (Shinkafi and Dauda, 2013). Polyphenols from plants have been reported to have antibacterial activity (Agarwal *et al.,* 2007). Also, the results of the anti-nutrient values may contribute to its medicinal value. Some of these compounds are well documented to exhibit hypoglyceamic activity in animals (Akhtar *et al*., 1981). Saponins inhibit Na^+ efflux leading to higher Na^+ concentration in cells, thereby activating a Na^+ - Ca^{2+} antiport (Schneider and Wolfing, 2004). This effect produces elevated cytosolic Ca^{2+} which strengthens the contraction of the heart muscle and thereby reducing congestive heart failure (Schneider and Wolfing, 2004). The low minimum inhibitory concentration (MIC) value observed is a good indication of high efficacy of the extracts against the test organisms; while high MIC may be an indication of low efficacy or that the organisms have the potential for developing resistance to the bioactive compounds. For the methanolic extract of the red onion, a minimum concentration of 25 mg/ml inhibited the *Staphylococcus aureus*, but all the different concentrations used have no effect on *Pseudomonas aeruginosa* while the ethanolic extract exhibited inhibitory effect on *Staphylococcus aureus* at a concentration of 12.5 mg/ml and 50 mg/ml was the minimum that inhibited

Table 4. Zone of inhibition of Ethanolic extracts of both white and red *Allium cepa* at various concentration on different isolates.

Extract concentration (mg/ml)	*Pseudomonas aerogenosa* (mm)		*Staphylococcus aureus* (mm)	
	White Onion	Red Onion	White Onion	Red Onion
50.0	28.19±0.043[a]	24.35±0.014[a]	34.79± 1.52[a]	29.81±0.022[a]
25.0	22.81± 2.000[b]	20.17±0.021[b]	27.38±0.021[b]	22.59±0.014[b]
12.5	16.42±0.07[c]	14.31±0.28[c]	20.58±0.07[c]	16.75±0.07[c]
6.25	0.00±0.00[d]	9.24±0.022[d]	11.32±0.014[d]	11.98±0.014[d]
Control	0.00±0.00	0.00 ± 0.00	0.00±0.00	0.00 ± 0.00
Ciproflaxacin	20.96±0.07	24.54±0.07	20.59±0.07	25.99±0.07

Values are means ± standard deviation, (n=3). Values followed by different letters are significantly different ($P < 0.05$) from each other for each isolates between the different extract concentration. n = number of replicates.

Table 5. Minimum Inhibitory concentration (mg/ml) of red onion for *Staphylococcus aueus* and *Pseudomonas aeruginosa.*

Organism	Ethanol (mg/ml)		Methanol (mg/ml)	
Staphylococcus	Red Onions	White Onions	Red Onions	White Onions
	12.5	6.25	25.0	25.0
Aureus				
Pseudomonas				
Aeruginosa	50.0	25.0	No growth	12.5

Pseudomonas aeruginosa. Also, the ethanolic extract of the white onions at 6.25 mg/ml showed inhibition on *Staphylococcus aureus* and 25.0 mg/ml inhibited the growth of *Pseudomonas aerugenosa,* while the methanolic extract inhibited *Pseudomonas aerugenosa* at 12.5 mg/ml and 25.0 mg/ml on *Staphylococcus aureus.* This suggests that *Staphylococcus aureus* was more sensitive to the extracts even at low concentration compared to *Pseudomonas aerugenosa,* Hence, comparing the antibacterial activity of the onion extracts with commercially available antibiotic (ciprofloxacin) used in this study, the results showed a significant difference ($p \leq 0.05$) in the values recorded for both extracts of white *Allum cepa* with higher values than the commercial antibiotics at the same concentration. There are abundant alternative orthodox therapeutic agents (antibiotic) around us than the constantly used synthetic drugs which have its side effect in the body and which the organisms usually end up becoming resistant to.

Conclusion

This study revealed that *Allium cepa* extracts have antimicrobial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa*, which can be attributed to the phytochemicals such as flavonoids, alkaloids, phenols, saponins and other secondary metabolites, present in the plant extracts. From the result obtained it clearly shows that the ethanolic extracts are more effective than the methanolic extracts to the test organisms.The minimum inhibitory concentration (MIC), result showed that the ethanolic extract of white onion have the least MIC value.

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