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Comparative Studies on Phytochemical Constituents and antimicrobial Activity on Three Onion Species

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Abstract: Several studies have been carried out on the phytochemical constituents of onions, without recourse to the understanding that there are different species of onions *(Allium cepa, Allium ascalanicum and Crinum ornatum).* This study is designed to determine and compare the phytochemical constituents and antimicrobial activity of different species of onions. *Phytochemical analysis was carried out on the different plant samples using distilled water, ethanol and chloroform* as solvents. The results gotten *show that flavonoid, saponin and steroids were present while tannin was present in the ethanol extract and absent in the rest extract. Glycosides were present in distilled and chloroform extracts, alkaloid was present in both distilled water and ethanol extracts but absent in the chloroform extract. Hydroxyl anthraquinone was absent in all three samples Phytochemical data for Crinum ornatum, indicated the absence of flavonoid, tannins and hydroxyl anthraquinone was absent in distilled water extract, for the ethanol extract flavonoid, tannins saponin, glycosides alkaloid were present while steroid and hydroxyl anthraquinones were absent. Flavonoid, saponin, glycosides steroid and hydroxyl anthraquinones were absent in the chloroform extract while tannins and alkaloids were present. The information obtained for their phytochemical analysis indicated that onion bulbs have some medicinal values. The antimicrobial investigations showed that the ethanol extracts of crinum ornatum, allium cepa and allium ascalonicumexhibited11.2, 8.9and 8.9mm which is consistent with the zone of high inhibition against the Staphylococcus aureus while the distilled water and chloroform extracts showed minimum inhibitory action. However, Efaecalis ethanol extracts of Allium cepa, Allium ascalanicum and cranium ornatum displayed the highest inhibitory action of 4.84.8 and 8.2 mm respectively for E. faecalis, compared to distilled water, ethanol. For E-colalsohighgh inhibitory zones exhibited ethanol extract of Crinum ornatum (8.6 mm), allium cepa (5.8mm) and Allium ascalonicum (5.8mm) were also observed., compared to distilled water and chloroform extracts that exhibited inhibitory zones positioned at 18and 4.4 and 4.4 mm for Crinum ornatum, Allium cepa and Allium ascalonicum respectively. Other active inhibitory zones were also observed and they gave evidence that the different species of onions have certain .antimicrobia properties*

Keywords: *Phytochemical, antimicrobial, Allium cepa, Allium ascalonicum and Crinum ornatum*

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1.0 Introduction

Human beings have depended on plants for survival since ever (Khan, *et al.*, 2017). Nature has blessed aerobic organisms with an inner defense system that resists oxidative damage due to reactive oxygen species (Khan *et al.,* 2013). It is well acknowledged that plants are the richest resorts of antioxidants. An exceptionally strong antioxidant, onion is the richest source of numerous compounds occupying the second position as an imperative vegetable grown and consumed all over the world (Ashwini & Sathishkumar, 2014). Allium species (onions) have been reportedly employed in the treatment of different kinds of ailments. Antioxidants comprising compounds in onions are tocopherols, alkaloids, flavonoids, glycosides carotenoids, phenolic compounds and acids (Khan, *et al.,* 2016). Compounds from onions such as sulfur, organo-sulfur, calcium and riboflavin have a range of health benefits such as anti-carcinogenic, anti-platelet, anti-thrombotic, anti-asthmatic, anti-diabetic, fibrinolytic, anti-helminthic, anti-inflammatory, antiseptic, antispasmodic, carminative, diuretic, expectorant, febrifuge, hypoglycemic, hypotensive, lithotriptic and hypocho-lesterolemic properties and other various biological actions including antibiotic effects (Ashwini &Sathishkumar, 2014).

The common onion (*Allium cepa*), which is also called a bulb onion and garden onion, is the most widely cultivated species of the genus allium. The genus *Allium* also contains several other species that are cultivated for food, such as the Japanese bunching onion (*allium fistulosum*), Egyptian onion (*A. ×proliferum*) and Canadian onion (*A. canadense*). The name wild onion is applied to severalAlliumspecies. The vast majority of cultivars of *A. cepa* belong to the 'common onion group' (*A. cepa var. cepa*) and are usually referred to simply as onions. The aggregate group of cultivars (*A. cepavar. aggregatum*) includes both shallots and bulb onions. In onion, sulphur is a constituent of secondary compounds, that is, all in, cycloallin and thiopropanol. These secondary compounds not only govern the taste, pungency and medicinal properties of onion but are also important for resistance against pests and diseases. In the overall

cropping pattern, onion occupies about 0.1% of the gross cropped area (area under all crops in the country) and about 7 percent of the total area under all vegetable crops. To determine the comparative studies on phytochemical constituents and antimicrobial activity on three onion species (Sharma, 2014).

2.0 Materials and Methods

2.1 Sample Collection and Preparation

Samples for the three different species of onions were collected from Kure market, In Minna, Niger state. The moisture-free samples were respectively crushed into powder form using wood mortar and pestle. The aqueous extract was prepared by soaking 100g of the dried powder into 500 cm^3 of distilled water for 24 hours and the filtrate obtained was preserved and used for phytochemical analysis. Similar methods were followed for the preparation of the methanolic extract, except that the solvent was replaced with methanol.

10% NaOH, 5% $FeCl_3$, 50% H_2SO_4, 10% HCl, Mayer's and Wagner;reagents were prepared using standard methods. However, in the preparation of Meyer's reagent, 13.35 g of mercuric chloride was dissolved in60 cm^3 of distilled water to give solution A. 5 g of potassium iodide was dissolved in 20 cm^3 of distilled water to give solution B. solutions A and B were mixed and transferred to 1000 cm^3 volumetric flask and made up to the mark with distilled water. Wagner's reagent was prepared by dissolving 1.27 g of a sublimed solution of iodine and 2 g of KI dissolved in 20 cm^3 of distilled water. The resultant solution was transferred into a 1000 cm^3 volumetric flask and made up to mark with distilled water.

2.2 Phytochemical screening of the extract

Qualitative tests were carried out on the aqueous extract and the powdered samples using standard procedures for identifying chemical constituents as described (Softwares 1993; Trease and Evans, 1989; Harborne, 1973).

2.2.1 Test for Flavonoids

3 cm^3 aliquot of the filtrate was added to 1 cm^3 of 10 % NaOH. The yellow colour displayed indicates the possible presence of flavonoid compounds.

2.2.2 Test for tannins

5% ferric chloride solution was added in drops to 2-3 cm^3 of the extract and the blue-black colour change was observed which indicates the presence of tannins.

2.2.3 Test for saponins

10 $cm^3$of the extract was placed into a test tube and mixed with 5 cm^3 of distilled water and shaken vigorously for stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of an emulsion.

2.2.4 Test for glycosides

25 cm^3 of 50 % H_2SO_4 was added to 5 cm^3 of the extract in a test tube. The mixture was heated in boiling water for 15 minutes, cooled and neutralized with 10 % NaOH. 5 cm^3 of Fehling solution was then added to the mixture and allowed to cool. A brick-red precipitate was observed which indicates the presence of glycosides.

2.2.5 Test for alkaloids

2 cm^3 of each extract was mixed with 2 cm^3 of 10% aqueous HCl. 1 cm^3 of the resultant mixture was treated with a few drops of Wagner's reagent for a second, whereas 1 cm^3 was treated similarly with Mayer's reagent. Turbidity or precipitation of either of these reagents was taken as preliminary evidence for the presence of alkaloids.

2.2.6 Test for steroids

50 cm^3 of the sample extract was dissolved in 2 cm^3 of chloroform and 25 cm^3 of H_2SO_4 was carefully added to form a lower layer. A reddish-brown colour at the interface was observed which indicates the presence of a steroidal ring.

2.2.7 Test for hydroxyl-anthraquinones

50 cm^3 of each of the plant extracts was mixed with 10 cm^3 benzene and 5 cm^3 of 10% NH_3

solution. The mixture was shaken and the pink, red or violet colour was observed at the ammoniacal flower) phase indicates the presence of anthraquinones.

2.3 Antimicrobial analysis

2.3.1 Determination of mini inhibitory concentration (MIC) using macro dilution method

The minimum inhibitory concentration of *V. amygdalina* and *occimum gratissimum* were determined based on the macro dilution method (Berghe&Vlitinck, 1991) with some modifications as follows:

The extracts were serially diluted (two folds) in a series of test tubes using nutrient broth supplemented with 10% glucose and 0.05% phenyl red indicator. These were later inoculated with 0.2M suspension of the test organism. The final concentrations were in the range of 1000 - 10 $ulml^{-1}$ in the medium. Microbial growth was determined by observing the colour change in the tube (red-yellow when there in growth). The lowest concentration that showed no change of colour was considered as the MIC.

2.3.2 Determination of antimicrobial activity

Cup-plate method using Muller-Hilton Ager medium was employed to study the preliminary antibacterial activity of *occimum gratissimum* extracts against different microbial strains. preparation of nutrients broth, sub-culture, base layer medium, agar medium &pepton water was done as per the standard procedure. The cups each of 9 mm diameter were made by scooping out medium with a sterilized cork borer in a petri dish that was stricken with the organisms. The *vernomia amygdalina* and *occimum gratissimum* extract (50 ul) were added separately in the cups and Petri dishes were subsequently incubated. Kanamycin was used (30 ug) as standard reference drugs. The zone of inhibition produced by the plant extracts was measured in mm.

3.0 Results and Discussions

The results of the phytochemical constituents of *Allium cepa, Crinium ornatum* and *Allium ascalonicum* are presented in Tables 1 and 2. indicating the presence of flavonoid, saponins, glycosides, alkaloids, and steroids. While hydroxyl-anthraquinones and tannins were observed to be absent. Similarly, the ethanol extract shows the absence of only hydroxyl-anthraquinones and glycosides, the chloroform extract of common onion indicates that tannins, alkaloid, and hydroxyl-anthraquinones were generally absent. These constituents are secondary metabolites so their presence in this plant will give the plant its medicinal value. The presence of steroids in this plant will give the plant a strong relationship as a sex hormone.

Table 1:Results from the preliminary qualitative phytochemical screening of *Allium cepa*

Test	Distilled water	Ethanol	Chloroform
Flavonoid	+	+	+
Tannins	-	+	-
Saponins	+	+	+
Glycosides	+	-	+
Alkaloids	+	+	-
Steroids	+	+	+
Hydroxyl anthraquinones	-	-	-

****; + present, _ absent**

Table 2: Results from the preliminary qualitative phytochemical screening of *Allium ascalonicum*

Test	Distilled water	Ethanol	Chloroform
Flavonoid	+	+	+
Tannins	-	+	-
Saponins	+	+	+
Glycosides	+	-	+
Alkaloids	+	+	-
Steroids	+	+	+
Hydroxyl anthraquinones	-	-	-

**** + present, _ absent**

Table 3: Results from the preliminary qualitative phytochemical screening of *Criniumornatum*

Test	Distilled water	Ethanol	Chloroform
Flavonoid	-	+	-
Tannins	-	+	+
Saponins	+	+	-
Glycosides	+	+	-
Alkaloids	+	+	+
Steroids	+	-	-
Hydroxyl anthraquinones	-	-	-

****; + present, _ absent**

The result in Table 3 indicated the phytochemical screening of *crinum ornatum* indicates the presence of saponins, glycosides, alkaloids and steroids in the distilled water extracts and the ethanol extract shows that steroids and hydroxyl-anthraquinone were absent while the chloroform extract of *Crinum ornatum* shows that tannins and alkaloid are present while other constituents were absent. *Crinum ornatum* phytochemical screening shows that flavonoid tannins and hydroxyl-anthraquinone were absent in distilled water extract, for the ethanol extract flavonoid, tannins, saponin, glycosides alkaloid were present while steroid and hydroxyl-anthraquinone were absent. Flavonoid, saponins, glycosides, steroids and hydroxyl-anthraquinones were absent for the chloroform extract while tannins and alkaloids were present. The presence of some of this metabolite constituent gives these onions their medicinal values.

The antimicrobial analysis of Tables 4, 5 and 6 shows that the ethanol extract has 11.2 mm, 8.9 and 8.9 mm which is a high inhibitory zone against the organism *Staphylococcus aureus* for *Crinum ornatum*, *allium cepa*, and *Allium ascalonicum*. The distilled water and chloroform extract showed minimum inhibitory action similarly, *E-faecalis* for ethanol extract *of Allium cepa*, *Allium ascalonicum* and *Crinum ornatum* show the highest action of 4.8, 4.8 and 8.2 mm respectively as compared to distilled water and ethanol. *E-coli* also shows a high inhibitory zone against ethanol extract having 8.6mm of C*rinum ornatum*, 5.8 mm for both *Allium cepa* and *Allium ascalonicum* as compared to distilled water and chloroform extract having

18 mm and 4.4 mm respectively have a minimum inhibitory zone. Ethanol extract against *K-pneumonia* of *Crinum ornatum* is 4.8mm and has no effect on *Allium cepa* and *Allium ascalonicum* as compared to distilled water and chloroform extract. Also, *P-aeroginosa* with 6.8mm has higher inhibitory action for ethanol extract of all the samples used. *Candida albicans* and *S-aureus* also show a similar yield of the high inhibitory zone for ethanol extract having 1.8 mm, 4.2 mm, 4.2 mm for *Crinum ornatum, Allium cepa*, *Allium ascalonicum* respectively.

Table 4: Micro-organisms/minimum inhibitory concentration (µg /ml) for common and shallot onion

Plants	Solvents used.	Staphylococcus aureus	E-F	E-C	K-P	P-A	C-A	S- aureus
Allium cepa	**Ethanol**	8.9	4.8	5.8	-	6.8	4.2	8.1
	Water	6.8	2.8	6.6	-	5.9	5.4	4.2
	chloroform	1.8	0.4	1.1	-	1.2	1.4	1.5
Allium ascalonicum	**Ethanol**	8.9	4.8	5.8	-	6.8	4.2	8.1
	Water	6.8	2.8	6.6	-	5.9	5.4	4.2
	chloroform	1.8	0.4	1.1	-	1.2	1.4	1.5

** EF= ***E-faecalis***, **E-C= *E-coli*, K-P= *K-pneumonie*, P-A = *P-aeroginosa*, C-A= *Candida albicans***

Table 5: Micro-organisms/minimum inhibitory concentration (µg /ml) for common and shallot onion

Plants	Solvents used.	Staphylococcus aureus	E-F	E-C	K-P	P-A	C-A	S-aureus
Allium ascalonicum	**Ethanol**	8.9	4.8	5.8	-	6.8	4.2	8.1
	Water	6.8	2.8	6.6	-	5.9	5.4	4.2
	chloroform	1.8	0.4	1.1	-	1.2	1.4	1.5

**** EF= **E-faecalis, E-C= E-coli, K-P= K-pneumonie, P-A = P-aeroginosa, C-A= *Candida albicans***

Table 6:Result of micro-organisms/minimum inhibitory concentration (µg/ml) of *Criniumornatum*

Plants	Solvents used	Staphylococcus aureus	E-F	E-C	K-P	P-A	C-A	S-aureus
Crinium ornatum	Ethanol	11.2	8.2	8.6	4.8	6.8	1.8	4.6
Crinium ornatnm	Water	1.8	1.6	1.8	1.8	2.9	1.4	3.5
Crinium ornatum	Chloroform	5.6	1.4	4.4	4.2	4.8	3.7	6.1

The result of antimicrobial screening of distilled water for *crinum ornatum*has 3.5 mm against *S-aureus* which has a high inhibitory action as compared with 1.8mm, 1.6mm,

1.8mm, 2.9 mm and 1.4 mm of *Staphylococcus aureus, E-faecalis, E-coli, K-pneumonie, P-aeroginosa, Candida albicans* respectively. A*llium cepa* and *Allium ascalonicum* exhibited inhibitory action that indicated *Staphylococcus aureus* with 1.8 mm as a high inhibitory action when compared with 0.4 mm for *E-faecalis*, 1.1 mm for *E-coli*, 1.2 mm for *P-aeroginosa*, 1.4 mm for C*andida albicans*, and 1.5mm for *S-aureus*. However, that of *K-pneumonie* was undetected. The chloroform extract of *Allium cepa* and *Al*lium *ascalonicum* shows that the inhibitory zone for *K –pneumonie* was not detected while *Staphylococcus aureus* exhibited 6.8 mm as the highest inhibitory action. Other organisms had a minimum inhibitory zone.

4.0 Conclusion

Considering the results of phytochemical and antimicrobial screening of common and shallot onions bulbs in comparison with *crinum ornatum* bulbs the result justifies that these onions could be considered as a stimulating source of flavoring and medical benefit when consumed.

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Conflict of Interest

The authors declared no conflict of interest