Screening of some Bioactivities and Investigation of some Chemical Constituents of *Allium cepa* Linn. (Onion bulb)

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Abstract

This research deals with the screening of antimicrobial and antioxidant activities and investigation of some chemical constituents of *Allium cepa* Linn. (Onion) bulbs.*Allium cepa* Linn. (Onion) bulbs have a long history of medicinal use. Onion is usually through of as a vegetable. The phytochemical investigation of onion bulbs indicated the presence of alkaloids, α-amino acids, carbohydrates,flavonoids, glycosides, phenolic compounds, reducing sugars whereas starch, tannins, steroids, terpenoids, saponins, cyanogenic glycosides and organic acids were not detected.Agar well diffusion method was used to determine in *vitro* antimicrobial activity on the different extracts (EtOAc, PE, MeOH, EtOH and water). Antioxidant activity of onion bulbs was also investigated by using DPPH radical scavenging assay. The IC_{50} of MeOH, EtOH, EtOAc and water extracts from onion bulbs were observed to be 2.33, 3.71, 4.28 and 6.46 μgmL^{-1}, respectively. The MeOH extractisthe most effective the other extracts. However, all extracts showed mild activity when compared to the standard antioxidant BHT (1.07 μgmL^{-1}). In chemical investigation, two compounds (yield percent 0.02 % and 0.04 %) were isolated from ethanol extract of onion bulbs. The isolated compounds were characterized by TLC, chemical tests, UV and FT IR spectroscopic techniques.

Keywords: *Allium cepa* Linn. (Onion), phytoconstituents, antimicrobial,antioxidant

Introduction

Onion (*Allium cepa*Linn.) is usually thought of as a vegetable. It also has a long history of medicinal use. Mainly the freshly bulb that grows below the ground is used medicinally as well as for food but other parts of the plant also has a place in the traditional medicines. The bulbs, tubers and rhizomes are able to survive under harsh conditions, *e.g*., winter and dryness. Onion bulb contains several oligosaccharides, Quercetin, anthocyanin, diallyl disulfide, flavonol glucoside dimer, Querrcetin-3,4´-oli *O*-b-glucosideand Querrcetin-4´-O-β-glucoside (Virginia, 2006, David, *etal*., 2010 and Freddy, *et al*., 2006).

Botanical Aspect of Onion (*Allium cepa* Linn.)

Botanical name	:	*Allium cepa* Linn.
Family	:	Alliaceae
Genus	:	*Allium*
Species	:	*cepa*
English name	:	Onion
Myanmar name	:	Kyet-thun-ni
Plant Parts used	:	Flower, leaf, seed and bulb
Distribution	:	Myanmar, Europe, Asia, North America and Africa

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Uses of Onion (*Allium cepa* Linn.)

The plant of onions are proved to show the antidiabetic, antioxidant, anti-hypertensive, anti-thrombotic, hypoglycemic, anti-hyperlipidemic (Dhanpra, 2007), antimicrobial and anti-allergic activities. Onions (*Allium cepa*) plant is used as traditional remedy in the treatment of various disorders so it has particular medicinal important (Kook, *et al*., 2009). Photographs of Oniun Bulbs are as shown in Figure 1.

(a) Whole plant of onion (b) Fresh bulb of onion (c) Fresh flower of onion

Figure 1. Photographs of *Allium cepa* Linn. (Onion)

Materials and Methods

The medicinal plant of onion (*Allium cepa* Linn.) bulbs was chosen to be studied in the present research because onion bulbs have antibacterial and antioxidant activities. The sample of onion (*Allium cepa* Linn.) bulbs were purchased from Hlaing market, Hlaing Township, originally from Magway Township, Magway Region. The plant of onion (*Allium cepa* Linn.) bulbs was identified in Department of Botany, University of Yangon, Myanmar. The collected bulbs sample was removed skins and cleaned by washing thoroughly with water and air-dried at room temperature. The dried samples were cut into small pieces and ground into powder by a grinding machine. The powder sample was obtained and stored in air-tight container to prevent other contamination.

Preliminary Phytochemical Test

A few gram of dried powder of onion was subjected to the tests of alkaloids, α-amino acids, carbohydrates, flavonoids, glycosides, organic acids, phenolic compounds, reducing sugars, saponins, starch, tannins, steroids, terpenoids according to the standard procedures. (Trease and Evans, 1980, Robinson, 1983, M-Tin Wa, 1970, Volgel, 1996, Harborne, 1984, Marini-Bettolo *et al.*, 1981)

Antimicrobial activity screening of onion bulbs

Antimicrobial property was studied on PE, EtOAc, 95 % EtOH, MeOH and H_2O extracts from the samples against 6 strains of microorganisms including *Bacillus subtilis, Bacillus pumilus, Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli* and *Candida albicans* by employing agar well diffusion method.

Screening of Antioxidant Activity by DPPH Assay Method

The DPPH radical scavenging method was used to evaluate the antioxidant property. The antioxidant activity was compared with that of the Butylated Hydroxyl Toluene (BHT). The concentrations of the crude extracts required to scavenge DPPH showed a dose dependent response. The antioxidant activity of each crude extract was expressed in terms of IC_{50}. The sample solution were prepared by mixing 1.5 mL of 0.002% (w/v) DPPH solution was mixed with 1.5mL of various concentrations (10, 5, 2.5, 1.25, 0.625µg/mL)of sample crude extracts. The reduction of the DPPH free

radical was measured by reading the absorbance at 517 nm by a UV-visible spectrophotometer (UV- 7504, KWF, China).

Separation and Isolation of some Constituents from Ethanol Extract Onion Bulbs

Ethanol extract from onion bulbs was firstly separated by applying silica gel column chromatographic method eluting with various ratios of PE : EtOAc,EtOAc : MeOH solvent system.

Characterization of isolated compounds by different reagents

The isolated compounds from *Allium cepa* Linn. (Onion) bulbs were checked by using TLC with different reagents: 5 % $FeCl_3$, Liberman, 1 % $AlCl_3$ and NH_3. And then, the isolated compounds were tested the following reagents: 5 % $FeCl_3$, 10 % NaOH, 1 % $AlCl_3$, 1 % HCl, 1 % KOH, Mg/HCl and 10 % lead acetate for flavonoids and glycosides by using Test Tube Methods.

Determination of Ultra Violet spectra

The UV spectra of isolated compounds were determined in methanol alone and by adding different shift reagents (NaOH, $AlCl_3$ and $AlCl_3$+ HCl). The spectra were recorded on Shimadzu UV-240, UV-visible spectrophotometer (Japan)at the Universities' Research Centre (URC).

Determination of FT IR spectrum

FT IR spectra of isolated compounds were recorded on a Shimadzu FT IR-8400 Fourier Transform Infrared Spectrometer at the Universities' Research Centre (URC), University of Yangon, Myanmar.

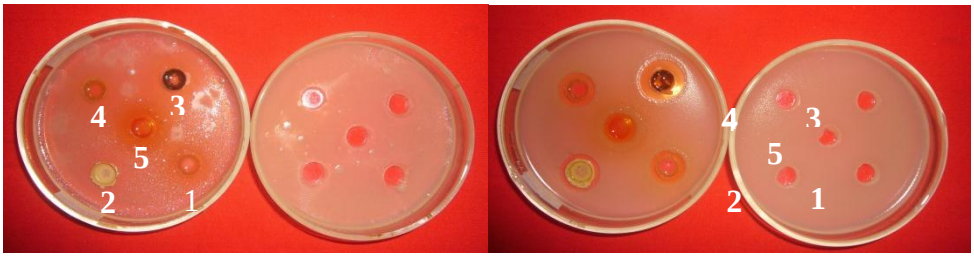
Results and Discussions

The phytochemical investigation of onion bulbs indicated the presence of alkaloids, α-amino acids, carbohydrates, glycosides, phenolic compounds, reducing sugars and flavonoids whereas starch, tannins, steroids, terpenoids, saponins, cyanogenic glycosides and organic acids were not detected.

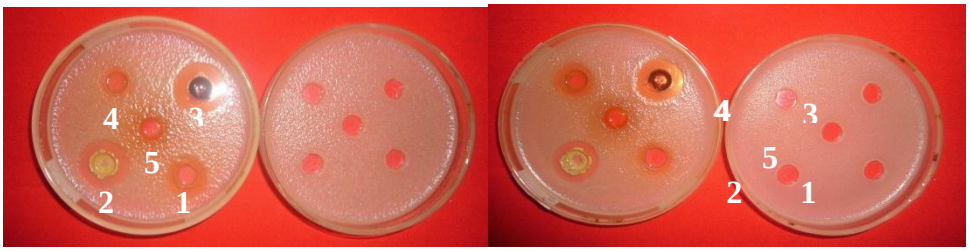
Antimicrobial activity of five extracts (PE, MeOH, EtOAc, EtOH and H_2O) obtained from *Allium cepa* Linn. (Onion) bulbs were investigated on 6 different strains of microorganisms such as *Bacillus subtilis, Bacillus pumilus, Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli*and *Candida albicans* by agar well diffusion method. The measureable zone diameter, including the well diameter, shows the degree of antibacterial activity. The well diameter is 10 mm in this experiment. The larger zone diameter has more activity on the test organisms. The photographs of illustrating the inhibiting zones provided by crude extracts against six species of microorganisms and observed data are summarized in Figure 2and Table 1.

Antioxidant activity of onion was investigated by using DPPH radical scavenging assay.The IC_{50}values for all crude extracts tested and standard BHT are tabulated in Table 2 and Figure 3. The IC_{50}ofMeOH,EtOH,EtOAc and H_2O extracts from Onion bulbs were observed to be 2.33, 3.71, 4.28 and 6.46 μgmL^{-1}, respectively. The IC_{50} values of MeOH extracts is lower than other extracts. So, the

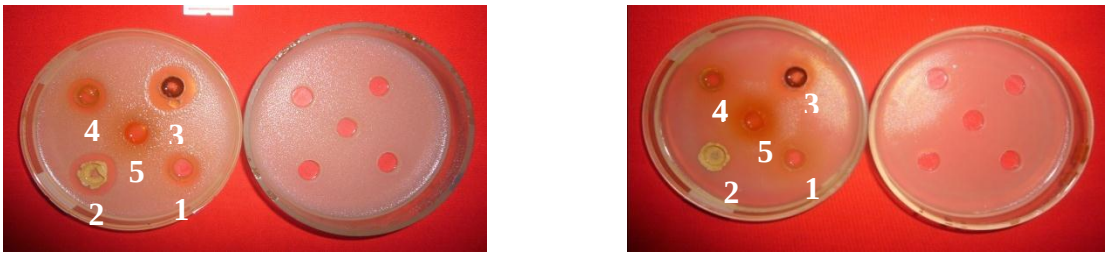
MeOH extractis more effective than other extracts. All extracts showed mild activity when compared to the standard antioxidant BHT (1.07 µgmL^{-1}).



Bacillus subtilis *Staphylococcus aureus*



Pseudomonas aeruginosa *Bacillus pumilus*



Candida albicans *Escherichia coli*

Figure 2.Antimicrobial activity of *Allium cepa* Linn. (Onion) bulbs

1 = Pet-ether extract 2 = MeOH extract 3 = EtOAc extract

4 = EtOH extract 5 = H_2O

Table 1.Results of Antimicrobial Activity Screening on *Allium cepa* Linn. (Onion) Bulbs

Organisms	Inhibition Zone Diameter of Extracts (mm)				
	PE	MeOH	EtOAc	EtOH	H_2O
Bacillus subtiils	13 (+)	17 (++)	20 (+++)	–	14 (+)
Bacillus pumilus	20 (+++)	14 (+)	20 (+++)	17 (++)	–
Staphylococcus aureus	18 (++)	15 (++)	20 (+++)	15 (++)	14 (+)
Pseudomonas aeruginosa	23(+++)	18 (++)	24 (+++)	14 (+)	–
Escherichia coli	–	–	15 (++)	14 (+)	–
Candida albicans	20 (+++)	15 (++)	23 (+++)	17 (++)	–

Diameter of agar well = 10 mm, 10 mm ~ 14 mm = (+)
15 mm ~ 19 mm = (++), 20 mm above = (+++)
No activity = (–)

Table 2. Percent Oxidative Inhibition of Various Concentrations and IC_{50} Values of Four Crude Extracts from Onion Bulbs and Standard BHT on Antioxidant Activity

Test samples	Percent Oxidative Inhibition in Different Concentration (µg/mL)					IC_{50} (µg/mL)
	0.625	1.25	2.5	5	10	
Water extract	20.96	26.77	39.47	47.38	56.37	6.46
EtOAc extract	21.21	30.56	42.10	53.17	61.49	4.28
EtOH extract	24.75	35.10	44.64	55.75	62.41	3.71
MeOH extract	36.36	41.92	51.26	63.13	67.93	2.33
Standard BHT	37.12	55.16	60.86	72.44	77.13	1.07

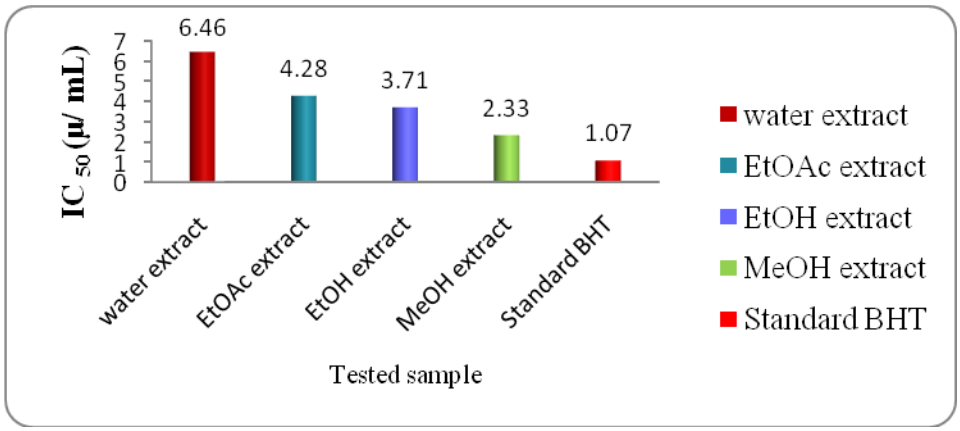


Figure 3.IC_{50} values of four crude extracts from onion bulbs and standard BHT

Separation and Isolation of some Constituents from Ethanol Extract Onion Bulbs

The compounds Aand B were isolated from ethanol extract of Onion bulbs by silica gel column chromatography using PE : EtOAc and EtOAc : MeOH solvent system. Two Isolated compounds A and Bwereobtained as an amorphous powder.

Characterization of isolated compounds

The isolated compounds were checked by thin layer chromatography. GF_{254}precoated silica gel aluminium plate (Merck) was used as adsorbent and EtOAc: MeOH (19 : 1) solvent system as mobile phase. The localization of spot was made by viewing directly under UV (254 nm and 365 nm wavelength) lamp. It was observed as a one spot.

Two isolated compounds were also spraying with 5 % $FeCl_3$, Liberman, 1 % $AlCl_3$and NH_3. The R_f value of Compounds Aand B were 0.43 and 0.33 in EtOAc : MeOH (19 : 1) solvent system. The pictures are shown in and Figure 5.

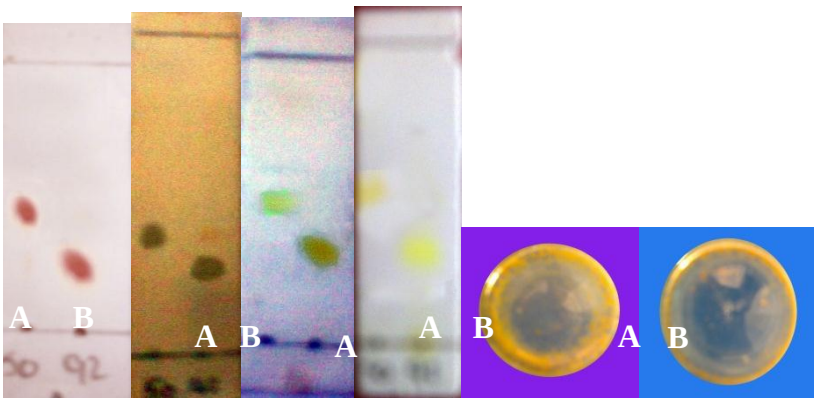
In addition the isolated compounds were checked by the following reagents: 5 % $FeCl_3$, 1 M KOH, 10 % NaOH, 1 % $AlCl_3$, 1 % HCl, Mg / HCl and 10 % lead acetate for flavonoids and glycosides by using Test Tube Methods. The isolated compounds may be flavonoids glycosides. The result data are shown in Table 3.

Determination of Ultra Violet spectra

UV spectra of isolated compound A and B are shown in Figure 5,6Table 4 and5. According to UV spectrum the major absorption bands were found to be 256 and 372nm. This information pointed out that compound A and compound B contain double bond conjugation.

Determination of FT IR spectrum

The functional groups present in compound A and B were also studied by FTIR spectroscopy as shown in Figure 6, Table6and 7.

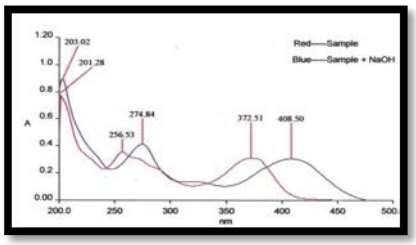


(a) Liberman **(b)**5 % FeCl **(c)**1 % $AlCl_3$**(d)** NH_3**(e)** **(f)**

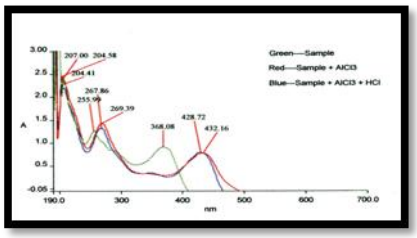
Figure 4. Isolated compounds by checking with TLC and crystallization with MeOH

Table 3. Chemical Tests of IsolatedCompound A and B by Different Reagents for Flavonoids and Glycosides (Test Tube Method)

Solvent	Reagent	Observation	Result
MeOH	5 % $FeCl_3$	Brown ppt	Phenolic group present
	1 M KOH	Bright yellow	Phenolic group present
	10 % NaOH	Yellow	Phenolic group present
	Mg turning / HCl	Pale pink	Flavonoids present
	1 % $AlCl_3$	Yellow green	Flavonoids present
	1 % HCl	Pale yellow	Flavonoids present
	10 % lead acetate	Orange ppt	Glycosides present



(a) Compound A with MeOH and NaOH

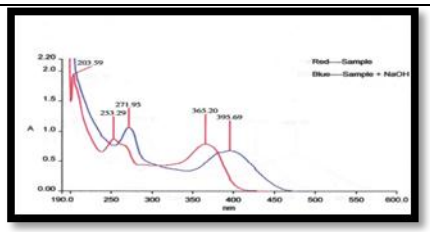


(b) Compound A with MeOH, $AlCl_3$ and HCl

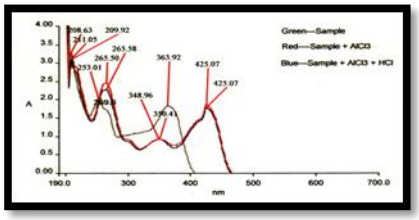
Figure 5. UV-visible spectra of isolated Compound A

Table 4. UV Spectral Data of Observed and Literature Values of Isolated Compound A

Solvent	λ_{max}/ nm				Interpretation
	Literature		Observation		
	Band II	Band I	Band II	Band I	
MeOH	250 – 280	350 – 385	257	373	Flavonols



(a) Compound B with MeOH and NaOH



(b) Compound B with MeOH, $AlCl_3$ and HCl

Figure 6.UV-visible spectra of isolated Compound B

Table 5. UV Spectral Data of Observed and Literature Values of Isolated Compound B

Solvent	λ_{max}/ nm				Interpretation
	Literature		Observation		
	Band II	Band I	Band II	Band I	
MeOH	250 – 280	350 – 385	253	365	Flavonols

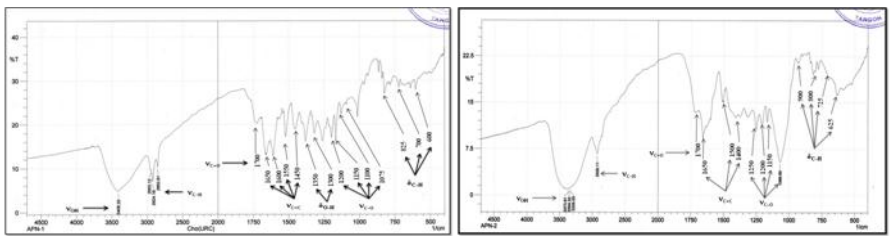


Figure 7.FT IR spectrum of isolated Compound A and B

Table 6.FT IR Spectral Data of Isolated Compound A

Wave number / cm^{-1}	Vibrational mode	Band assignment
3408	ν_{O-H}	hydroxy group
2953, 2924 and 2853	ν_{C-H}	aliphatic $-CH_2$, $-CH_3$
1700	$\nu_{C=O}$	chelated C=O
1650,1600, 1550 and 1450	$\nu_{C=C}$	aromatic ring
1350, 1300	δ_{O-H}	OH bending vibration of phenol group
1200,1150, 1100 and 1075	ν_{C-O}	phenolic group
825 – 600	δ_{C-H}	methyl group

Table 7. FT IR Spectral Data of Isolated Compound B

Wave number / cm^{-1}	Vibrational mode	Band assignment
3354	ν_{O-H}	OH group
2926	ν_{C-H}	CHasymmetric and symmetric
1700	$\nu_{C=O}$	chelated C=O
1650, 1500 and 1400	$\nu_{C=C}$	aromatic group
1250, 1200, 1150 and1068	ν_{C-O}	phenolic group
900 – 625	δ_{C-H}	methyl group

Conclusion

In the present research work, the following inference may be deduced from the overall assessment.

The phytochemical investigation of onion bulbs indicated the presence of alkaloids, α-amino acids, carbohydrates, glycosides, phenolic compounds, reducing sugars and flavonoids whereas starch, tannins, steroids, terpenoids, saponins, cyanogenic glycosides and organic acids were not detected.

According to antimicrobial activity screening, the EtOAc extract of bulbs exhibited the highest activity against all tested microorganisms. The PE extract and MeOH extract of the bulbs sample showed the antibacterial activity against *Bacillus subtilis, Bacillus pumilus, Pseudomonas aeruginosa, Staphylococcus aureus* and *Candida albicans.* The four extract of EtOH was showed the antibacterial activity against *Pseudomonas aeruginosa, Bacillus pumilus, Staphylococcus*

aureus,Escherichia coli and *Candida albicans*. Finally, the water extract of the bulbs sample showed the antibacterial activity against *Bacillus subtiils* and *Staphylococcus aureus*.

From the screening of free radical scavenging activity by DPPH assay on four extracts: MeOH,EtOH, EtOAc and H_2O extracts from Onion bulbs and compared with standard BHT. It was found that the IC_{50}valueofMeOH,EtOH, EtOAc and H_2O extracts from Onion bulbs were observed to be 2.33,3.71, 4.28 and 6.46 μgmL^{-1}, respectively. Test results revealed that MeOH extract is more effective than other extracts. Standard BHT (Butylated Hydroxy Toluene), synthetic antioxidant was used as a standard reference which showed IC_{50} value, 1.07 µg/mL. Test samples of *Allium cepa*Linn. (Onion) bulbs showed mild activity when compared to synthetic antioxidant.

The isolated two compounds (yield percent 0.02 % and 0.04 %) were also isolated from ethanol extract of onion bulbs. Isolated compounds were characterized by chemical tests, TLC, UV and FT IR spectroscopic techniques. According to the chemical test and spectroscopic data, the two isolated compounds showed the presence of aliphatic group, chelating carboxyl group, hydroxyl group especially phenolic–OH and $–CH_2OH$ group. According to the check by different reagents, two isolated compounds may be flavonols glycosides.

Therefore, it can be inferred that since *Allium cepa* Linn. (Onion) bulbs showed good antibacterial and antioxidant activity.In addition, it may be also contributed they can be effectively used as antibacterial agent as well as antioxidant in the treatment of inflammation, oxidative stress related diseases, some forms of cancer and many age-related disorders drug in Myanmar Traditional medicine.

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