Phytochemical Screening, Antioxidant Activity and Antimicrobial Activity in the Fruit of *Cucumis Melo* Var. *Reticulatus* L. Naud. (Muskmelon)

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Abstract

In this research work, the fruit of Cucumis melo var. reticulatus L. Naud. (Muskmelon) was selected for chemical analysis. The muskmelon sample was collected from Tan Soe village, Kyaukse Township, Mandalay Region. Firstly, phytochemical screening of the selected sample was determined by using standard method and the result revealed that phenolic, polyphenols, flavonoids, saponins, glycosides and reducing sugars were observed in this sample. The physical properties of the selected sample such as pH value (6.4), moisture (18.0 %), ash (9.8 %) contents were also determined. In addition, mineral contents was determined by Energy Dispersive X-ray Fluorescence (EDXRF) spectrophotometer. It was found that many elements were present in muskmelon, among than potassium, chlorine, calcium, copper, iron, zinc and sulphur. Moreover, the antioxidant activity of ethanol extract of this sample was measured by using 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay method using ascorbic acid as standard. From the experimental analysis the IC_{50} value of standard ascorbic acid was 5.01 µg/mL and ethanol extract of muskmelon sample was found to be 680.13 µg/mL. The antimicrobial activity of crude extracts of muskmelon was carried out by agar well diffusion method in various solvent systems on six selected microorganisms such as *Bacillus subtilis*, *Candida albicans*, *E. coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Salmonella typhi*. Inhibitation zone of ethyl acetate crude extract was more effective activity than other extracts.

Keywords: *Cucumis melo* var. *reticulatus* L. Naud. (Muskmelon), phytochemical screening, antioxidant and antimicrobial activities

Introduction

Medicinal plants are the prominent source of therapeutic agents used to prevent the human pathogenic bacteria and fungus. *Cucumis melo* L. (*Reticulatus* group), commonly called as cantaloupe or muskmelon, is a member of the *Cucurbitaceae* family (Bailey,1976). Consumer preference for this fruit is determined largely by its sweetness (i.e sugar content), flavor or aroma, texture and more recently as a rich source of phytonutrients (Lester, 2008). Muskmelon is one of the top ten fruit crops grown widely in the world for their delicious sweetness, high nutrient quality and flavor (Cheng ZJ, *et al*., 2007). They contain biological active components known as phytochemicals that promote health through the prevention of specific degenerative diseases such as cardiovascular diseases, diabetes, obesity, cancers and gastrointestinal tract disorders. Antioxidant can be gained from food processing industries especially agricultural by products (Aleksandra, (2007) DC).

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These by products are promising sources of important bioactive substances called phytochemicals (carotenoids, phenolics, and flavonoids), antioxidants and vitamins that possess beneficial physiological properties (Lachman, 2013 and Yildrim, 2001). Moreover, little information is known regarding the correlation between the bioactive compounds and antioxidant activity in muskmelon fruit.

Botanical Ascept of *Cucumis melo* var. *reticulatus* L. Naud.

Figure 1. Fruit and plant of muskmelon

Scientific name	-	Cucumis melo var. Reticulatus L. Naud.
Family	-	Cucurbitaceae
English name	-	Muskmelon
Myanmar name	-	Tha-khwa-hmwei
Part Used	-	Fruit pulp

Materials and methods

Collection of Samples

The muskmelon sample was collected from Tan-Soe Village, Kyaukse Township, Mandalay Region. The fruit was washed with water and then the peels of the skin were removed. The flesh was cut into slices and dried in the shade at room temperature. The air-dried sample was used for analysis.

Determination of Phytochemical Screening on Muskmelon Sample

Phytochemical screening of muskmelon was carried out by the standard methods (Harborne, 1998).

Determination of pH in Muskmelon Sample

pH of muskmelon sample was determined by portable pH meter (Ip67 water proof, thermo Scientific, U.S. A)

Determination of Moisture and Ash Content in Muskmelon Sample by AOAC

Moisture content in dried sample was determined by oven-drying method (Herbert Weisz, 2013) and the ash content of sample was determined by the use of muffle-furnace method.

Determination of Elemental Analysis of Muskmelon Samples

Element contents in muskmelon sample was determined by using (Energy Dispersive X-ray Fluorescence Spectroscopy) EDXRF at University of Mandalay.

Determination of Antioxidant Activity in Muskmelon Samples by DPPH Assay Method

Dried powders (50 g) of the fruit of muskmelon were percolated in 150 mL of ethanol at room temperature for about six weeks. Filtration gave the ethanol soluble filtrate and residue. The filtrate was evaporated to get ethanol extract.

In this research work, investigation of antioxidant activity of ethanol extract from fruit of muskmelon was performed by DPPH assay. Ethanol extract was prepared and the radical scavenging activity was evaluated. The antioxidant power is expressed as IC_{50} (50 % inhibitory concentration). It is the test substance concentration (μg/mL) that results in a 50 % reduction of initial absorbance of DPPH solution.

About 2 mg of the ethanol extract to be tested were dissolved in 95 % ethanol and made the volume up to 10 mL. The solution was then filtered to get a stock solution.The desired concentrations of test sample solutions (50, 100, 200, 400, 600, 800 μg/mL) were prepared by diluting the stock solution with 95 % ethanol.

Procedure

(i) Control solution was prepared by mixing 60 μM DPPH solutions 2 mL and 95 % ethanol 2 mL.

(ii) Similarly, the blank solution was prepared by mixing 2 mL of test solution and 95 % ethanol 2 mL.

(iii)The sample solution was prepared by mixing 2 mL of test solution with 60 μM DPPH solution.

All these solutions were allowed to stand at room temperature for 30 minutes. Then, the absorbance was measured at λ 517 nm using spectrophotometer UV-160 IPC (P/N 206-6750), Shimadzu Corporation.

Absorbance of individual solution was measured and calculated the % inhibition. Then IC_{50} value was calculated by linear regressive excel program.

Determination of Antimicrobial Activity in Muskmelon Sample by using Agar Well Diffusion Method

Glucose 0.5 g, yeast extract 0.3 g, peptone 0.3 g, agar1.7 g and 100 mL of distilled water were added in a 250 mL sterile conical flask and heated on hot plate until boil medium. Then, the mouth of the flask was plugged with a piece of cotton wool. This medium was sterilized in an autoclave at 121˚C for 45 minutes. After 45 minutes, 0.1 mL test organisms were inoculated into 20 mL of medium agar at about 40 ˚C and were poured into the sterile petri- dishes at aseptic condition. After the agar become solid, cock borer was used to make the wells (8 mm in diameter). Then, extract samples (20 μL) were introduced into the well and they were incubated at room temperature for 24-48 hours. After 24-48 hours of incubation, the clear zones were measured. Clear zone surrounding the wells indicated the presence of antimicrobial active compound in the extracts which inhibit the growth of the test organisms.

Test Organisms

Bacillus subtilis (Fever), *Candida albicans* NITE 09542(Skin disease), *Escherichia Coli* AUH5436 (Diarrhea, abdominal pain, Pneumoinia), *Pseudomonas aeruginosa* (Urinary tract infection, dermatitis), *Staphylococcus aureus* AUH 8456

(food Poisoning), *Salmonella typhi* (Typhoid Fever) were used for agar well diffusion method. These test organisms were obtained from National Institute of Technology and Evaluation (NITE), Japan and Pharmacological Research Department (PRD), Yangon, Myanmar.

Results and discussion

Determination of Phytochemical Screening on Muskmelon Samples

The results of preliminary photochemical screening of muskmelon sample were shown in Table 1.

Table 1. Preliminary Phytochemical Screening of *Cucumis melo* var. *reticulatus* L. Naud. (Muskmelon)

Test	Extract	Observation	Remark
Phenolic	EtOH	purple color solution	+
Polyphenols	EtOH	orange color solution	+
Flavonoids	EtOH	yellow color solution	+
Saponins	H_2O	frothing	+
Glycosides	H_2O	white ppt	+
Reducing Sugars	H_2O	deep orange ppt	+
Alkaloids	1.5 % HCl	orange ppt	-
Terpenes	EtOH	red color solution	+

EtOH=ethanol (+) =presence
ppt=precipitate (-) =absence

Figure 2. Phytochemical screening of *cucumis melo* var. *reticulatus* L. Naud. (Muskmelon)

According to the results, the fruit of *Cucumis melo* var. *reticulatus* L. Naud. It consists of phenolic, polyphenols, flavonoids, saponins, glycosides, reducing sugars and terpenes. It may be the source of antioxidant and have antimicrobial activity. Phytochemical polyphenols have received great attention because of their biological activity. The having many flavonoids in fruit sample, it was shown that strong antioxidant properties (Saidu and Garb, 2011)

Determination of pH Value, Moisture and Ash Content of Muskmelon Sample

Physical and chemical properties of selected sample such as pH, moisture and ash contents are shown in Table 2.

Table 2. pH value, Ash and Moisture Content of Muskmelon

Sample	pH	Moisture (%)	Ash (%)
Muskmelon	6.4	18.0	9.8

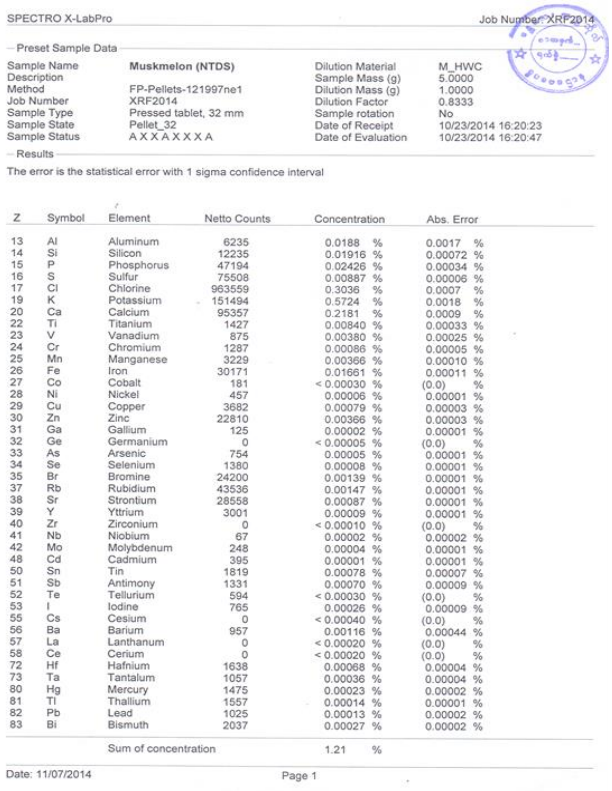
According to this table, moisture content is one of the most important and widely used analytical measurements in the food products. The moisture content of muskmelon samples was found to be 18.0 %. Therefore, water content of muskmelon fruit may be fairly high and it can be used for human diet. The ash percent is the measure of the quality of food; the higher ash percent the better is the food quality. Since the ash content of muskmelon sample was found to be 9.8 %, it can be said that muskmelon fruit has good food quality.

Determination of Elemental Analysis of Muskmelon Samples

Elemental compositions of muskmelon were determined by source excited Energy Dispersive X-ray Fluorescence method (EDXRF) at University of Mandalay. The results were shown in Table 3.

Table 3. Elemental Analysis of Muskmelon by Energy Dispersive X-ray Fluorescence (EDXRF)

Elements	Relative content (%)
Potassium	0.5724
Chlorine	0.3036
Calcium	0.2181
Copper	0.1760
Iron	0.0166
Zinc	0.0110
Sulphur	0.0088



SPECTRO X-LabPro Job Number: XRF2014

Preset Sample Data

Sample Name	**Muskmelon (NTDS)**	Dilution Material	M_HWC
Description		Sample Mass (g)	5.0000
Method	FP-Pellets-121997ne1	Dilution Mass (g)	1.0000
Job Number	XRF2014	Dilution Factor	0.8333
Sample Type	Pressed tablet, 32 mm	Sample rotation	No
Sample State	Pellet_32	Date of Receipt	10/23/2014 16:20:23
Sample Status	A X X A X X X A	Date of Evaluation	10/23/2014 16:20:47

Results

The error is the statistical error with 1 sigma confidence interval

Z	Symbol	Element	Netto Counts	Concentration	Abs. Error
13	Al	Aluminum	6235	0.0188 %	0.0017 %
14	Si	Silicon	12235	0.01916 %	0.00072 %
15	P	Phosphorus	47194	0.02426 %	0.00034 %
16	S	Sulfur	75508	0.00887 %	0.00006 %
17	Cl	Chlorine	963559	0.3036 %	0.0007 %
19	K	Potassium	151494	0.5724 %	0.0018 %
20	Ca	Calcium	95357	0.2181 %	0.0009 %
22	Ti	Titanium	1427	0.00840 %	0.00033 %
23	V	Vanadium	875	0.00380 %	0.00025 %
24	Cr	Chromium	1287	0.00086 %	0.00005 %
25	Mn	Manganese	3229	0.00366 %	0.00010 %
26	Fe	Iron	30171	0.01661 %	0.00011 %
27	Co	Cobalt	181	< 0.00030 %	(0.0) %
28	Ni	Nickel	457	0.00006 %	0.00001 %
29	Cu	Copper	3682	0.00079 %	0.00003 %
30	Zn	Zinc	22810	0.00366 %	0.00003 %
31	Ga	Gallium	125	0.00002 %	0.00001 %
32	Ge	Germanium	0	< 0.00005 %	(0.0) %
33	As	Arsenic	754	0.00005 %	0.00001 %
34	Se	Selenium	1380	0.00008 %	0.00001 %
35	Br	Bromine	24200	0.00139 %	0.00001 %
37	Rb	Rubidium	43536	0.00147 %	0.00001 %
38	Sr	Strontium	28558	0.00087 %	0.00001 %
39	Y	Yttrium	3001	0.00009 %	0.00001 %
40	Zr	Zirconium	0	< 0.00010 %	(0.0) %
41	Nb	Niobium	67	0.00002 %	0.00002 %
42	Mo	Molybdenum	248	0.00004 %	0.00001 %
48	Cd	Cadmium	395	0.00001 %	0.00001 %
50	Sn	Tin	1819	0.00078 %	0.00007 %
51	Sb	Antimony	1331	0.00070 %	0.00009 %
52	Te	Tellurium	594	< 0.00030 %	(0.0) %
53	I	Iodine	765	0.00026 %	0.00009 %
55	Cs	Cesium	0	< 0.00040 %	(0.0) %
56	Ba	Barium	957	0.00116 %	0.00044 %
57	La	Lanthanum	0	< 0.00020 %	(0.0) %
58	Ce	Cerium	0	< 0.00020 %	(0.0) %
72	Hf	Hafnium	1638	0.00068 %	0.00004 %
73	Ta	Tantalum	1057	0.00036 %	0.00004 %
80	Hg	Mercury	1475	0.00023 %	0.00002 %
81	Tl	Thallium	1557	0.00014 %	0.00001 %
82	Pb	Lead	1025	0.00013 %	0.00002 %
83	Bi	Bismuth	2037	0.00027 %	0.00002 %
		Sum of concentration		1.21 %	

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Figure 3. EDXRF spectrum of fruit of muskmelon

It was found that potassium (0.5724 %) is the highest content and followed by chlorine (0.3036 %), calcium (0.2181 %), copper (0.1760 %), iron (0.0166 %), zinc (0.0110 %) and sulphur (0.0088 %). Potassium possesses hypertension activity and also it can reduce the levels of bad cholesterol in the blood.

Determination of Antioxidant Activity in Muskmelon Sample

The result of the absorbance, % inhibition of different concentration of ethanol extract and that of ascorbic acid was shown in Tables 4, 5, 6 and Figure 4,5.

Table 4. Absorbance Values and % Inhibition of Muskmelon Fruit Sample

Extract	Concentration (µg/mL)	Absorbance	% Inhibition
Ethanol extract	50	0.365	17.26
	100	0.363	20.13
	200	0.350	27.65
	400	0.260	30.75
	600	0.245	42.78
	800	0.148	64.36

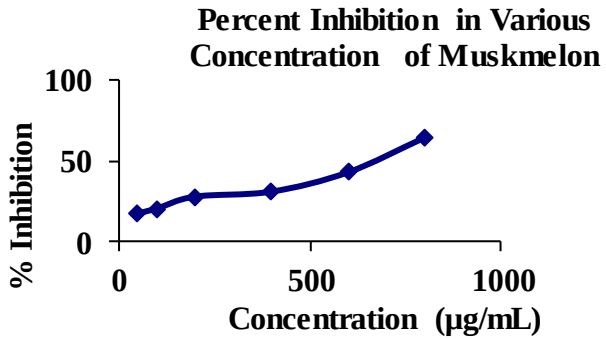

Figure 4. Percent inhibition in various concentration of *Cucumis melo* var. *reticulatus* L. Naud. (Muskmelon)

Table 5. Absorbance Values and % Inhibition of Standard Ascorbic Acid

Concentration (µg/mL)	Absorbance	% Inhibition
1.00	0.443	7.322
2.00	0.405	15.270
4.00	0.284	40.585
8.00	0.086	82.008
16.00	0.077	83.890

Table 6. IC_{50} Values of Ascorbic acid and Ethanol Crude Extract

Samples	IC_{50} (µg/mL)
Ascorbic Acid	5.01
Ethanol Crude Extract	680.13

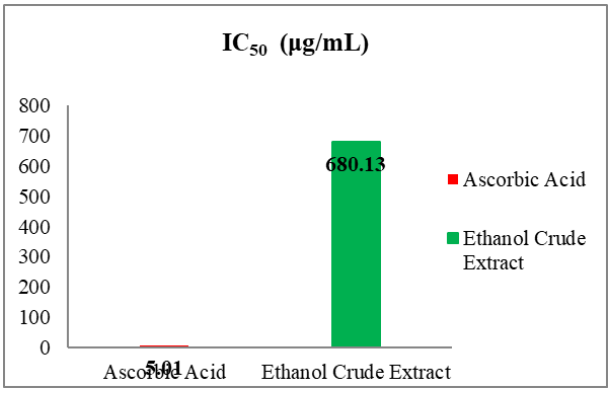

Figure 5. Comparison of IC_{50} values of standard ascorbic acid and ethanol crude extract of muskmelon

The IC_{50} value of muskmelon is 680.13 µgmL^{-1}. The IC_{50} value of standard ascorbic acid was 5.01 µgmL^{-1}. (Henane, 2023) reported that the antioxidant activities were detected in muskmelon with 283.63 µgmL^{-1} and 160.92 µgmL^{-1}.

Therefore, the selected muskmelon has slightly antioxidant activity because chemical compositions of muskmelon depend on the variety condition and nature of soil and climate.

Determination of Antimicrobial Activity in Muskmelon Sample by using Agar Well Diffusion Method

The results of extracted muskmelon were tested on six bacterial strains. The inhibition zones were described in Table 7.

Table 7. Antimicrobial Activity of *Cucumis melo* var. *reticulatus* L. Naud. (Muskmelon)

Extracts	Inhibition Zone (mm)					
	Bacillus Subtilis	*Candida albican*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Salmonella typhi*
H_2O	-	17.60	17.01	16.65	14.87	16.87
EtOH	17.01	16.96	13.35	12.10	12.90	18.11
EtOAc	30.51	26.74	32.65	34.93	35.14	32.10
PE	-	-	-	-	-	-

Agar well = 8 mm (20µL / well) (-) No Activity, (+) 9-14 mm, Low activity, (++) 15-20 mm, Medium activity, (+++) 21 mm-above, High activity

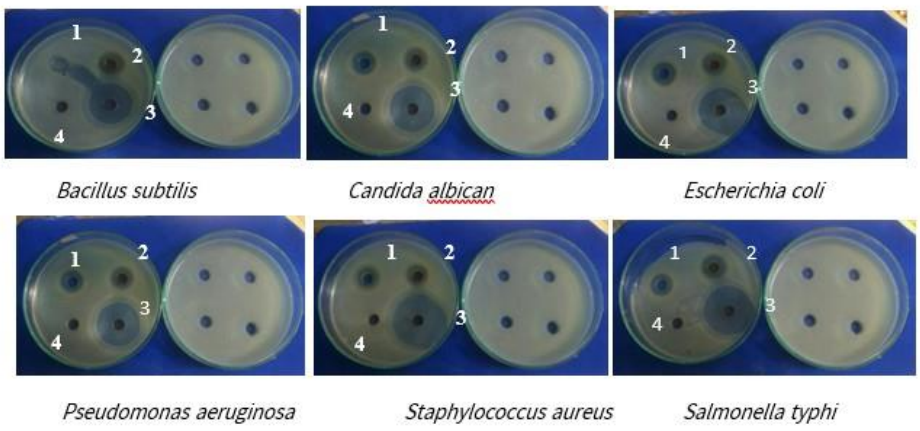

Figure 6. Antimicrobial activity of crude extracts of sample with various solvents

1=H_2O extract, 2= EtOH extract, 3=EtOAc extract,4= PE extract

In the results of antimicrobial activity of muskmelon, ethylacetate extract showed higher activity 30.51 mm against *Bacillus subtilis*, 26.74 mm against *Candida albicans*, 32.65 mm against *Escherichia coli*, 34.93 mm against *Pseudomonas aeruginosa*, 35.14 mm against *Staphylococcus aureus* and 32.10 mm against *Salmonella typhi* than ethanol extract (12.10 mm -18.11 mm) against on six test organisms. But petroleum ether extract was not shown antimicrobial activity in Table 8 and Figure 7. *Staphylococcus aureus* bacteria are very adaptable, and many varieties have become resistant to one or more antibiotics. *Escherichia coli*, no current treatments can cure the infection, relieve symptoms or prevent complications. *Candida albicans* Vaginal candidiasis is usually treated with antifungal medicine. *Bacillus subtilis* is not a frank human pathogen, but has on several occasions been

isolated from human infections. Infections attributed to *Bacillus subtilis* include bacteremia, endocarditis, pneumonia, and septicemia. So, muskmelon can give good health for the human body. These antimicrobials have played an essential role in the prevention, treatment, and control of food animal diseases caused by pathogens (Hoflack, G, 2001).

Conclusions

The fruit of *Cucumis melo* var. *reticulatus* L. Naud. (Muskmelon) possessed phytochemical, mineral elements which are required in the human body. The IC_{50} value of muskmelon is lower than that of standard ascorbic acid. Therefore, muskmelon has slightly antioxidant activity. The determination of antimicrobial activity of muskmelon, ethylacetate extracts showed higher than other extracts on six tested organisms. Fruit of *C. melo* var. *reticulatus* L. Naud. (Muskmelon) against broad range of disease-causing microorganisms. Thus, fruit of muskmelon can be used as antimicrobial agents in drugs for the therapy of infectious disease as caused by human pathogens. Chemical compositions of muskmelon depend on the variety and nature of soil and climate.

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