Investigation on the Nutritional values, Antimicrobial Activity and Antioxidant activity from Seed of *Ginkgo biloba* Linn.

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Abstract

In this research work, seed of *Ginkgo biloba* Linn. collected from local market, Mandalay, Mandalay Region was selected for the chemical investigations. Preliminary phytochemical investigations of the seed of *Ginkgo biloba* were tested to study the phytochemical constituents. It consists of alkaloid, flavonoid, steroid, terpene, polyphenol, glycoside, phenolic, tannin, saponin, lipophenol, reducing sugars and carbohydrate. And then, the nutritional values such as moisture, ash, fat, fiber, protein and carbohydrate of the seed of *Ginkgo biloba* were determined. In addition, the mineral contents of the seed of *Ginkgo biloba* were also detected by using EDXRF method. Furthermore, the antimicrobial activities of seed of *Ginkgo biloba* were determined by agar-well diffusion method on six selected organisms namely *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus pumilus*, *Candida albicans* and *Escherichia*- coli. The vitamin C content of the seed of *Ginkgo biloba* was investigated by using iodometric titration method. Finally, the antioxidant activity of the seed of *Ginkgo biloba* was determined by using DPPH Radical Scavenging Assay .

Keyward: *Ginkgo biloba*, phytochemical, nutritional values, mineral, antimicrobial, vitamin C, antioxidant

Introduction

Plants are the basis of healthy life. Most plants grow from seeds .Seeds are eaten in small quantities nutrients however they are rich in nutrient. *Ginkgo biloba* Linn. or maidenhair, is a tree native to china that has been grown for thousands of years for a variety of uses. The tree is widely cultivated in human history. It grows throughout China, Korea, Japan, Europe and the United states (Bradly p.Jacobs, 2018). Fresh *ginkgo* seeds are available in Japan and China and sold in markets. They are imported into some Western countries and sold in Chinese food stores, natural food stores or supermarkets. *Ginkgo biloba*, rich in antioxidants and other nutrients, improve heart health, promote brain function, reduce the depression, and improve eyesight. The unique health benefits of *ginkgo* also include its ability to improve cognition and treat Alzheimer's disease. (John Staughton, 2019).

Botanical Description (Ansley Hill, 2018)

Family name	–	Ginkgoaceae
Botanical name	–	*Ginkgo biloba* Linn.
Myanmar name	–	Gaba – Oo – Bin
English name	–	Maidenhair
Chinese name	–	Bai – Guo
Part of use	–	seed

Figure 1. Tree and Seeds of *Ginkgo biloba* Linn.

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Materials and Methods

Sample Collection

The seed of *Ginkgo biloba* Linn. to be analyzed were collected from local market, Mandalay, Mandalay Region. The sample was then dried in shade, cut into pieces and grounded by electric blender. It was stored in a well-stoppered bottle and used throughout the experiment.

Figure 2. Dried and Powder of Seed of *Ginkgo biloba* Linn**.**

Preliminary Phytochemical Investigation of Seed of *Ginkgo biloba* Linn.

The seeds of *Ginkgo biloba* Linn. were tested by phytochemical screening. (Harbone, J.B., 1973).

Determination of Moisture Contents of the Seed of *Ginkgo biloba* Linn (AOAC, 2000)

The sample (1 g) was placed in pre-weighed porcelain crucible. Then it was kept in an oven at 100°C for 30 min. It was cooled in desiccator and then weighed again. The process of heating, cooling and weighing was repeated until a constant weight was obtained.

Determination of Ash Contents of the Seed of *Ginkgo biloba* Linn. (AOAC, 2000)

The air dried sample (2 g) was weighed and placed in a preheated, cooled and weighed crucible. The crucible was heated carefully in the furnace at 550°C for 2 hours, burned off without flaming or until all the carbon was eliminated. When the materials were converted to white ash powder, the crucible was cooled at room temperature, in a desiccator and weighed again.

Determination of Fat Content of the Seed of *Ginkgo biloba* Linn. (AOAC, 2000)

50 g of sample powder was introduced into a thimble and placed in a soxhlet apparatus. Then the apparatus was fixed with round-bottomed flask (1000 mL) containing 250 mL of petroleum ether (b.p 60-80°C). The extraction flask was heated on a water bath for 12 hours. After extraction, the thimble containing the meal cake was placed in an oven until no odour of ether remains.

Determination of Crude Fibre Contents of the Seed of *Ginkgo biloba* Linn.

(Kenneth Helrich, 1990)

About 2 g of the defatted sample was placed into a 500 mL flask and then 200 mL of 1.25 % sulphuric acid solution was added and digested for about 30 minutes and then filtered. The insoluble residue was washed with the hot water in order to free from acid. Then the residue was washed down into the flask with 200 mL of 1.25 % sodium hydroxide solution and boiled for 30 minutes. After boiling, the residue was filtered again and washed with 15 mL of 95 % ethanol. After washing the residue was heated in an oven at 100°C until the constant weighed was obtained. Finally, the substance in the crucible was incinerated at a dull red heat for about 20 minutes until the carbonaceous matter had been removed.

Determination of Protein Contents of the Seed of *Ginkgo biloba* Linn. by using Kjeldahl's Method (Digestion, Distillation and Titration)

About 0.5 g of defatted sample, 5 g of annular sodium sulphate, 0.25 g of anhydrous copper II sulphate and 12.5 mL of 98 % sulphuric acid were added into Kjeldah's digesting flask. The flask was shaken until the contents were thoroughly mixed and it was heated till the mixture became green colour solution. The digested solution with 25 ml of boric acid was poured into the flask together with 50 mL of 40 % sodium hydroxide to make mixture strongly alkaline. The evolved ammonia was distilled off.

The distillilate was titrated with standard sulphuric acid solution, using 0.2% methyl red and methylene blue (2 drops) as an indicator. A blank determination was carried out.

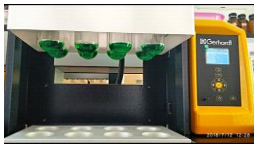


Figure 3. Digestion Set , Distillation Machine and Titration Set (Gerhardt, Germany)

Determination of Water-Soluble Carbohydrate Content of the Seed of *Ginkgo biloba* Linn. (James N.*et al*., 2009).

1 mL of sample solution and six standard sugar solutions containing 10, 20, 40, 60, 80 and 100 µg of glucose per mL were put in each test tubes. 1 mL of 5 % phenol solution was also added to each test tubes and mixed. A blank solution also prepared with 1 mL of distilled water instead of sugar solution. 5 mL of 96 % sulphuric acid was again added to each tube. Each test tube was agitated during the addition of acid. After 10 minutes, the tubes were reshaken and placed in water bath at 25°C-30°C for 20 minutes. The yellow orange colour solution was stable for several hours. Absorbances were measured at 490 nm using UV-visible spectrophotometer.

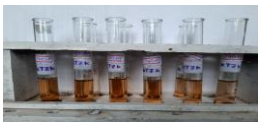



Figure 4. Sample Solutions and UV-Visible Spectrophotometer

Determination of Elemental Contents from the Seed of *Ginkgo biloba* Linn.

Elemental analysis of the seed of *Ginkgo biloba* Linn. was measured by Energy Dispersive X-rays Fluorescence (EDXRF) method at Department of Chemistry, Monywa University.

Determination of Antimicrobial Activities of the Seed of *Ginkgo biloba* Linn.

The antimicrobial activities of various extracts of *Ginkgo biloba* Linn. were done by Agar-well diffusion method on six selected organisms in Central Research and Development Centre (CRDC), Insein, Yangon.

Determination of Vitamin C Content by using Iodometric Titration Method of the Seed of *Ginkgo biloba* Linn.

25 mL of fresh juice sample was taken into a 250 mL conical flask. 10 drops of 1 % starch indicator solution was added and then titrated with standard iodine solution until blue-black colour solution was appeared.

Determination of Antioxidant Activity of the Seed of *Ginkgo biloba* Linn

In this experiment, 1-1 diphenyl -2- picrylhydrazyl (DPPH) powder was used as stable free radical. Ascorbic acid was used as standard antioxidant and ethanol (analar grade) was used as solvent. The absorbances were determined at 517 nm wavelength. (Manzocco, *et al.*, 1998).

Results and Discussion

Preliminary Phytochemical Test of Seed of *Ginkgo biloba* Linn.

Table 1. Results of Preliminary Phytochemical Constituents of Seed of *Ginkgo biloba* Linn.

No.	Constituents	Extract	Reagents used	Observation	Result
1.	Alkaloid	1 % HCl	Mayer's reagent	Cream ppt	+
2.	Flavonoid	95 % ethanol	Conc: HCl, Mg	Pink colour solution	+
3.	Steroid	95 % ethanol	Acetic anhydride, conc: H_2SO_4, $CHCl_3$	Green colour solution	+
4.	Terpene	95 % ethanol	$CHCl_3$, conc: H_2SO_4	Pink colour ppt	+
5.	Polyphenol	95 % ethanol	1 % $FeCl_3$ + 1 % $K_3[Fe(CN)_6]$	Greenish blue colour solution	+
6.	Glycoside	water	10 % lead acetate	White ppt	+
7.	Phenolic compound	water	10 % $FeCl_3$	Greenish blue colour solution	+
8.	Tannin	water	10 % $FeCl_3$, dil H_2SO_4	Yellowish brown ppt	+
9.	Saponin	water	shake	Froth	+
10.	Lipophenol	water	NaOH, 0.5 M KOH	Deep colour solution	+
11.	Reducing sugar	water	Benedict's solution	brick red ppt	+
12.	Carbohydrate	ethanol	Fehling's (A) + (B)	reddish brown colour	+

(+) = presence of constituents (–) = absence of constituents

According to this table, the seed of *Ginkgo biloba* Linn. consists alkaloid, flavonoid, steroid, terpene, polyphenol, glycoside, phenolic compound, tannin, saponin, lipophenol, reducing sugar and carbohydrate.

Determination of Moisture, Ash, Fat, Fiber, Protein and Carbohydrate Contents of Seed of *Ginkgo biloba* Linn.

Table 2. Results of Moisture Contents of Seed of *Ginkgo biloba* Linn.

No. of experiment	Wt. of sample (g)	loss of weight (g)	Moisture (%)
1.	1.00	0.0728	7.28
2.	1.00	0.0764	7.64
3.	1.00	0.0764	7.64

According to this table, the moisture content was 7.64%. It is a well - known fact that the higher the moisture content the greater the chances for the growth of microorganisms but if the moisture content is lower than about 12% the chances for growth of microorganisms are greatly minimized.

Table 3. Results of Ash Contents of Seed of *Ginkgo biloba* Linn.

No. of experiment	Wt. of sample (g)	Wt. of residue (g)	Ash (%)
1.	2.00	0.09	4.5
2.	2.00	0.07	3.5
3.	2.00	0.07	3.5

According to this table, the ash content was 3.5%. The ash content is a measure of the quality of food. The smaller the ash content the better will be food quality (Jenkins, Christian and Hager, 1953).

Table 4. Results of Fat Contents of Seed of *Ginkgo biloba* Linn.

No.	Wt. of sample (g)	Wt. of Fat (g)	Fat content (%)
1.	50.0000	5.2283	10.46
2.	50.0000	5.2394	10.48
3.	50.0000	5.2283	10.46

According to the experiment, the fat content was 10.46%. Fat is an essential part of our diet. It provides energy, absorbs certain nutrients and maintains our core body temperature.

Table 5. Results of Crude Fibre Contents of Seed of *Ginkgo biloba* Linn.

No.	Wt. of sample (g)	Wt. of fibre (g)	Crude fibre (%)
1.	2.00	0.0608	3.04
2.	2.00	0.595	2.98
3.	2.00	0.0595	2.98

According to this table, the fibre content was 2.98%. Fibers can help prevention of heart disease, diabetes, weight gain and some cancers, and can also improve digestive health.

Table 6. Results of Nitrogen and Protein Contents of Seed of *Ginkgo biloba* Linn.

No.	Nitrogen content (%)	Protein content (%)
1.	0.8112	5.070
2.	0.8112	5.070
3.	0.8113	5.071

This table showed that, protein content was 5.07%. Proteins need for a healthy and productive life. In the present work, the proteins (%) of the seed of *Ginkgo biloba* Linn. were calculated by multiplying the % nitrogen with the factor 6.25.

Table 7. Absorbance of Standard Glucose Solution

Concentration of Glucose (µg/mL)	Absorbance at 490 nm
10	0.363
20	0.467
40	0.569
60	0.700
80	0.838
100	0.972

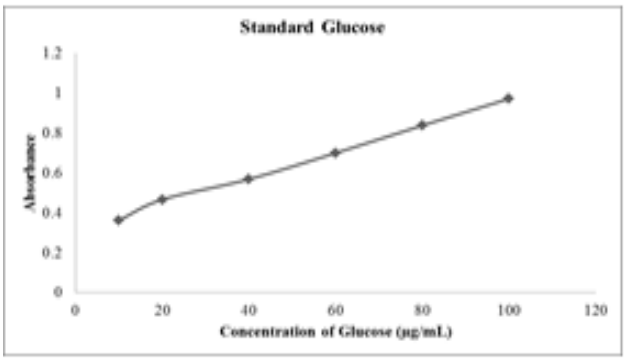


Figure 5. Standard Calibration Curve for Water Soluble Carbohydrate (As Glucose)

Table 8. Results of Soluble Carbohydrate Contents of Seed of *Ginkgo biloba* Linn.

No.	Soluble carbohydrate content (%)
1.	4.1
2.	4.2
3.	4.1

In the determination of carbohydrate, carbohydrates content was 4.1 %. Carbohydrates play a major role in promoting health fitness, form a major part of food and help a great deal in building body strength, by generating energy.

Elemental Composition of Seed of *Ginkgo biloba* Linn. by EDXRF Spectroscopy

Table 9. shows that some of elements (K, P, S, Fe, Cu, Zn) were found in the seed of *Ginkgo biloba* Linn.. Among them, potassium is the highest percent in this sample

Table 9. The Results of Elemental Content of Seed of *Ginkgo biloba* Linn.

Elements	Relative Abundance (%)
Potassium	0.665
Phosphorus	0.212
Sulfur	0.122
Iron	0.002
Copper	0.002
Zinc	0.001

Figure 6. Elemental Contents of Seed of *Ginkgo biloba* Linn. by EDXRF Spectroscopy

Antimicrobial Activities of Seed of *Ginkgo biloba* Linn.

The extracts of seed of *Ginkgo biloba* Linn. with various solvents such as n-hexane, ethyl acetate and ethanol were taken and examined the antimicrobial activities.

Table 10. Antimicrobial Activities of Seed of *Ginkgo biloba* Linn.

Sample	Solvents	Inhibition zone Diameter (mm)					
		I	II	III	IV	V	VI
Ginkgo	n-hexane	-	-	-	-	-	-
	EtOAc	-	-	-	20(+++)	-	-
	EtOH	_-	14 (+)	30(+++)	13(+)	12(+)	12 (+)

Agar-well ~ 10 mm

10 mm ~ 14 mm (+)

15 mm ~ 19 mm (++)

20 mm above (+++)

(–) = absent

Organisms

I = *Bacillus subtilis*

II = *Staphylococcus aureus*

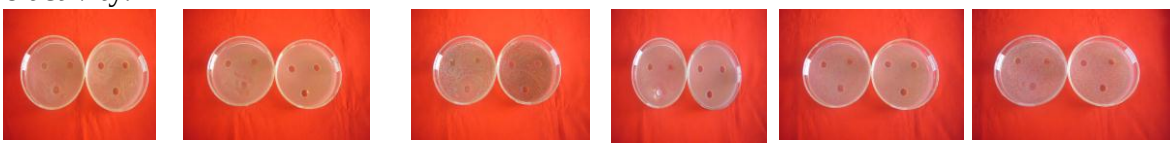
III = *Pseudomonas aeruginosa*

IV = *Bacillus pumilus*

V= *Candida albicans*

VI= *Escherichia-coli*

According to the experimental data, EtOAc extract of *Ginkgo biloba* Linn. showed high activity on *Bacillus pumilus*. Moreover, EtOH extract responded high activity on *Pseudomonas aeruginosa* and low activity on *Staphylococcus aureus, Bacillus pumilus, Candida albicans and Escherichia-coli.* n-hexane extract showed no activity.



Bacillus subtilis | *Staphylococcus aureus* | *Pseudomonas aeruginosa* | *Bacillus pumilus* | *Candida albicans* | *Escherichia-coli*

Figure 7. Antimicrobial Activities of Seed of *Ginkgo biloba* Linn.

Determination of Vitamin C Content of Seed of *Ginkgo biloba* Linn.

Table 11. Results of the Titration of 25 mL Fresh Juice Sample of Seed of *Ginkgo biloba* Linn. with Iodine Solution (Indicator-starch)

No.	Initial volume (mL)	Final volume (mL)	Volume used (mL)
Rough	0	3	3
1.	3	5.6	2.6
2.	5.6	8.3	2.7
3.	8.3	11.0	2.7

Calculation of Vitamin C Content

Titration of sample juice with I_2 solution

Juice (25 mL) = I_2 (2.7 mL) × 0.0076 M

= 0.02014 mmole of I_2

= 0.02014×10^{-3} mol × 176 $gmol^{-1}$

= 0.0036 g of ascorbic acid

Sample juice 100 mL = $\frac{100 \times 0.0036}{25}$ = 0.0144 g = 0.0144×10^3 mg = 14.4 mg

Content of ascorbic acid = 14.4 mg/100 mL

According to the experiment, the amount of ascorbic acid (vitamin C) in *Ginkgo biloba* Linn. was 14.4 mg/100 mL of the fresh sample.

Determination of Antioxidant Activity of the Standard Ascorbic Acid and Ethanol Extract of Seed of *Ginkgo biloba* Linn. by DPPH Radical Scavenging Assay

Table 12. % Inhibition of Various Concentrations of Standard Ascorbic Acid

Sample Concentration (µg/mL)	Mean Absorbance	Mean % inhibition	IC_{50} (µg/mL)
100	0.056	85.93	9.49
50	0.098	75.38	
25	0.149	62.56	
12.5	0.195	51.01	
6.25	0.234	41.21	

IC_{50} value was calculated by using linear regressive equation.

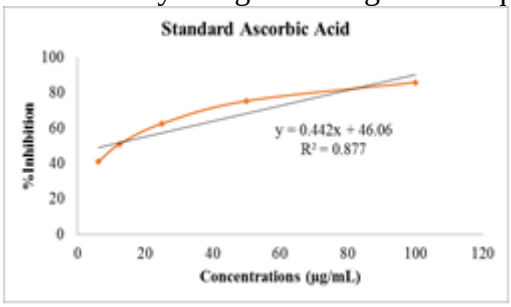


Figure 8. Plot of % Inhibition Vs Concentration of Standard Ascorbic Acid

Table 13. % inhibition of Various Concentrations of the Seed of *Ginkgo biloba* Linn.

Sample Concentration(µg/mL)	Mean Absorbance	Mean % inhibition	IC_{50} (µg/mL)
100	0.138	73.71	26.92
50	0.261	50.28	
25	0.265	49.52	
12.5	0.296	47.24	
6.25	0.283	46.14	

IC_{50} value was calculated by using linear regressive equation.

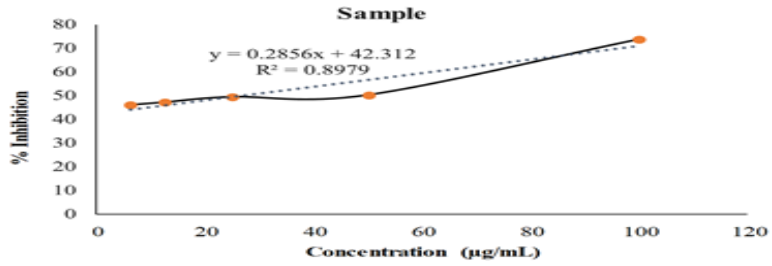


Figure 9. Plot of % Inhibition Vs Concentration of Seed of *Ginkgo biloba* Linn.

According to the experiment, the antioxidant activity of Seed of *Ginkgo biloba* Linn. was determined in DPPH free radical scavenging assay and IC_{50} value of Seed of *Ginkgo biloba* Linn. was found to be 26.92 µg/mL. It was higher than that of standard ascorbic acid (IC_{50} 9.49 µg/mL). So, the sample extract was lower antioxidant activity than standard ascorbic acid.

Conclusion

From the preliminary phytochemical investigation of the seed *Ginkgo biloba*, *it* consists of alkaloid, flavonoid, steroid, terpene, polyphenol, glycoside, phenolic, tannin, saponin, lipophenol, reducing sugar and carbohydrate. The nutritional composition of the seed of *Ginkgo biloba* Linn such as moisture (7.64 %), ash (3.5 %), fiber (2.98 %), fat (10.46 %), protein (5.070 %) and carbohydrate (4.1 %). The elemental contents of *Ginkgo biloba* were determined by EDXRF method. The amount of potassium was the highest percent (0.665%) in this sample. Moreover, antimicrobial activities of the seed of *Ginkgo biloba* Linn. in three solvents were also determined. Ethyl acetate extracts responds high activity on *Bacillus pumilus*. Moreover, ethanol extracts respond high activity on *Pseudomonas aeruginosa* and low activity on *Staphylococcus aureus, Bacillus Pumilus, Candida albicans and Escherichia*-coli. n-hexane extracts showed no activity. The vitamin C content of *Ginkgo biloba* Linn. was 14.4 mg/100 mL.. The IC_{50} values of ethanol extract of the *Ginkgo biloba* Linn. was found to be 26.92 µg/mL. It was higher than that of standard ascorbic acid (IC_{50} = 9.49 µg/mL). So, the seed of *Ginkgo biloba* Linn. extract has lower antioxidant activity than standard ascorbic acid. From the experimental data, the seed of *Ginkgo biloba* Linn. contains valuable chemical constituents and nutrients for human.

ACKNOWLEDGEMENTS

We would like to express our gratitude to Rector, Dr Thida Win, University of Mandalay for her interest and encouragement on our research work. We deeply thank to Dr Yi Yi Myint, Professor and Head, Department of Chemistry, University of Mandalay for her permission and facilities to do her patient guidance and invaluable advice. We also thank to Dr Khaing Khaing Kyu ,Professor, Department of Chemistry, University of Mandalay for her patient kind and invaluable suggestions during this research work.

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