

Preliminary Phytochemical Screening and Antioxidant Activity on Fruits of *Amomum subulatum* Roxb.

No Ko Soe¹, May Phyo Thynn²

Abstract

This research deals with the investigation of the biochemical properties, nutritional value and antioxidant activity of fruits from *Amomum subulatum* Roxb. belonging to the family Zingiberaceae. The fruit specimens were collected from Kachin State, Myitkyina District, Sadone Township at the altitude of (2000) M.A.S.L in July 2024. The preliminary screening analysis of the dried powdered fruits from *A. subulatum* Roxb. revealed the presence of the phytochemical elements such as alkaloids, steroids, terpenoids, glycosides, phenolic compounds, flavonoids, tannins, saponins, α -amino acid, proteins, reducing sugars, starch, and carbohydrates. Cyanogenic glycosides were not found in this study. The nutritional composition of *A. subulatum* Roxb. fruits were evaluated for moisture, total ash, crude fat, crude fiber, crude protein, carbohydrates, and energy content. The antioxidant activity of *A. subulatum* Roxb. was determined by 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay with different concentrations of watery fruit extracts. The result showed very strong antioxidant activity of fruits of *A. subulatum* Roxb. as indicated by a low IC₅₀ value of 11.9 ppm (11.9 μ g/ml). In conclusion, the fruits of *A. subulatum* Roxb. demonstrated the potential therapeutic properties although further research is needed. This study was believed to contribute to the development of natural drugs, food industries using as spices and to provide scientific evidence supporting these applications.

Keywords: Phytochemical, Nutritional, Antioxidant, Spices, Fruits, *Amomum subulatum* Roxb.

Introduction

Herbal drugs have been a fundamental component of traditional medicine systems worldwide and used since ancient times to treat a wide variety of diseases and ailments. Their effectiveness in promoting health and healing is attributed to the presence of complex chemical compounds. As a result, medicinal plants have played a vital role in global health promotion. It is estimated that approximately 25% of modern medicines are sourced, either directly or indirectly, from higher plants (Khan *et al.* 2024).

Amomum subulatum Roxb. belonging to the family Zingiberaceae is a cultivated large herb. It is commonly known as Hpala-gyi in Myanmar and Greater cardamon in English (Kress *et al.* 2003). *A. subulatum* Roxb. cultivated in the Sadone area is well-known for its exceptional flavor and is regarded as one of the best varieties of spices by local people. It is native to Eastern Himalayas, Nepal and India. It is also commercially cultivated in Nepal, India, China, and Bhutan, primarily in marginal, sloping, and degraded lands at elevations between 700 and 2100 meters. The cultivation occurs in regions with temperatures ranging from 4°C to 20°C and annual rainfall between 2000 to 2500 mm (Shrestha 2017).

A. subulatum Roxb. has been widely used in traditional medicine across various cultures, especially in South Asia. Its diverse medicinal applications are attributed to the health benefits derived from its fruits, seeds, and essential oils. It has been reported to possess beneficial effects as digestive disorders, respiratory ailments, oral health, infections, urinary disorders, nausea and vomiting, stress and anxiety (Bisht 2011, Satyal *et al.* 2012).

¹ Demonstrator, Department of Botany, Sagaing University

² Lecturer, Department of Botany, University of Mandalay

Herbs and plants are the base of various traditional medication systems practiced all over the world. Phytochemicals are present in plants which are secondary metabolites such as phenols, alkaloids, steroids, flavonoids, glycosides, saponins, anthocyanins, coumarins and certain pigments (Mehvish & Muhammad 2017). The phytochemical analysis is very important to evaluate the possible medicinal utilities of a plant and also to determine the active principles responsible for the known biological activities exhibited by the plants (Shaikh & Patil 2020).

An antioxidant is a molecule that prevents the oxidation of other molecules. Oxidation is a chemical process where electrons or hydrogen atoms are transferred from one substance to an oxidizing agent, often resulting in the formation of free radicals. These free radicals can trigger chain reactions that, when occurring within cells, may lead to cellular damage or death. Antioxidants interrupt these chain reactions by neutralizing free radical intermediates and inhibiting further oxidation processes. They achieve this factor by undergoing oxidation themselves, which is why many antioxidants act as reducing agents, such as thiols, ascorbic acid, or polyphenols (Shekhar & Prasad, 2015). By neutralizing free radicals, antioxidants help protect cells from damage. Free radical-induced reactions have been linked to various diseases, including cardiovascular disorders, diabetes, inflammatory diseases, and cancer (Sharma *et al.* 2016).

Plant-based foods include a diverse range of fruits, vegetables, whole grains, and their derived products. These foods serve as excellent sources of essential proteins, vitamins, and minerals, while also being abundant in various phytochemicals such as polyphenols, flavonoids, isothiocyanates, carotenoids, alkaloids, and terpenes (Xue & Yin 2024).

A. subulatum Roxb. is a spice that contains a wealth of essential nutrients and bioactive compounds, which make it an important ingredient in both culinary and medicinal uses. Although consumed in small amounts, it provides considerable nutritional and health benefits. Its blend of vital nutrients, dietary fiber, and bioactive compounds makes it a beneficial addition to a well-rounded diet (Dhakal *et al.* 2022).

In Myanmar, a variety of plant-based traditional medicines were used with ethnobotanical knowledge documented for many plant species. However, these practices still require further scientific evidence. Due to the traditional use of *Amomum subulatum* Roxb. and the lack of scientific evidence supporting its pharmacological properties, this study sought to evaluate the phytochemical composition and antioxidant activity of *A. subulatum* Roxb fruits. It was hoped that the investigations from this research would contribute to the development of natural medicines and support the use of plant-based resources in the food industries.

Materials and Methods

This research was conducted at Botany Laboratory in University Research Center (URC), University of Mandalay from July to November, 2024.

Plant Identification

The plant specimens were collected from Kachin State, Myitkyina District, Sadone Township in July, 2024. The collected specimens were studied and identified at the Department of Botany, University of Mandalay with the help of available literatures, Hooker (1894), Backer & Brink (1968) and Dassanayake (1983).

Preparation of Dried Powdered Fruit Samples

The fresh fruits of *Amomum subulatum* Roxb. belonging to the family Zingiberaceae were collected from Kachin State, Myitkyina District, Sadone Township in July, 2024. Freshly collected fruits were washed thoroughly and air-dried for several days

until completely dry. The dried fruits were then ground using a grinder, and the resulting powder was stored in an airtight container for subsequent analysis (Figure 1).

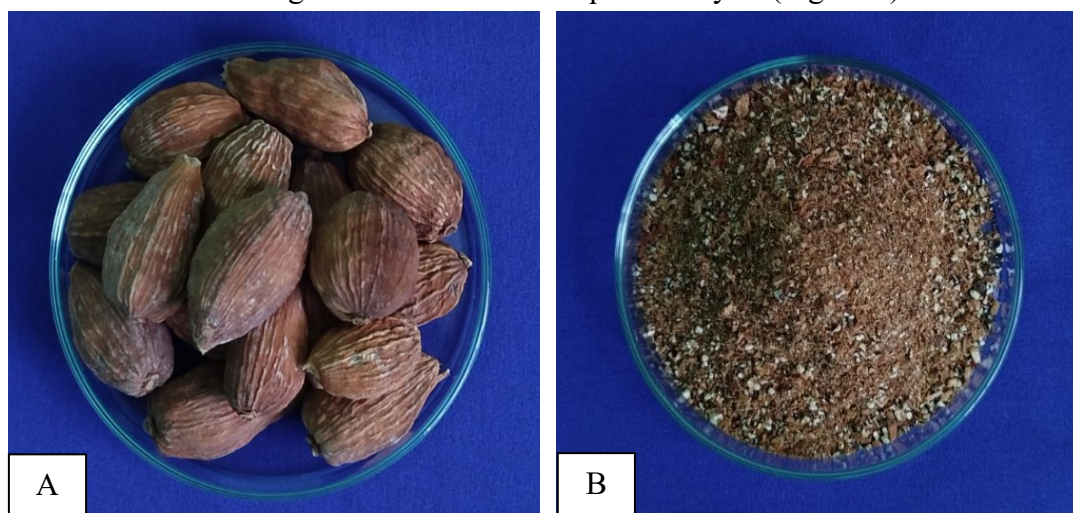


Figure 1. Fruit of *Amomum subulatum* Roxb.

- A. Air-dried Fruits
- B. Dried Powdered Fruits

Phytochemical Analysis

The phytochemical constituents including alkaloids, steroids, terpenoids, glycosides, phenolic compounds, flavonoids, tannins, saponins, α -amino acids, proteins, reducing sugars, starch, and carbohydrates were analyzed by using dried powdered fruits of *A. subulatum* Roxb. This analysis was conducted in October 2024 at the Botany Laboratory, University Research Center (URC), University of Mandalay. The phytochemical screening followed the standard protocol reported by Harborne (1998) with some modifications.

Antioxidant Activity and Nutritional Analysis

The antioxidant activity and nutritional value of the crude extract from the fruits of *A. subulatum* Roxb. were evaluated by using the DPPH free radical scavenging assay reported by Sharma & Bhat (2009). This analysis was carried out at U Kar Ka Food Testing Laboratory in Chanmyatharsi Township, Mandalay Region.

Results

Phytochemical analysis of Fruit Extracts of *Amomum subulatum* Roxb.

The phytochemical constituents such as alkaloids, steroids, terpenoids, glycosides, phenolic compounds, flavonoids, tannins, saponins, α -amino acid, proteins, reducing sugars, starch, carbohydrates and cyanogenic glycosides were analyzed. All of these constituents except cyanogenic glycosides were detected in the fruit extracts of *A. subulatum* Roxb (Table 1, Figure 2, Figure 3 and Figure 4).

Table 1. Preliminary phytochemical analysis of fruit extracts of *Amomum subulatum*

No	Tests	Extracts	Observation	Result
1	Alkaloids (Mayer's reagent)	Ethanol/Methanol/Watery/	White ppt.	+
	Alkaloids (Dragendorff's reagent)	Ethanol/Methanol/Watery	Grey	+
	Alkaloids (Wagner's reagent)	Ethanol/Methanol/Watery	Red ppt.	+
2	Steroids	Ethanol/Methanol/Watery	Blackish at interface	+
3	Terpenoids	Ethanol/Methanol/Watery	Yellowish green at lower layer	+
4	Glycosides	Ethanol/Methanol/Watery	Yellow ppt.	+
5	Phenolic compounds	Ethanol/Methanol/Watery	Blackish	+
6	Flavonoids	Ethanol/Methanol/Watery	Colourless	+
7	Tannins	Ethanol/Methanol/Watery	Blackish	+
8	Saponins	Ethanol/Methanol/Watery	No frothing/ frothing	+
9	α -amino acid	Ethanol/Methanol/Watery	Purple spot	+
10	Proteins	Ethanol/Methanol/Watery	White ppt.	+
11	Reducing Sugars	Ethanol/Methanol/Watery	Green ppt.	+
12	Starch	Ethanol/Methanol/Watery	Brown	+
13	Carbohydrates	Ethanol/Methanol/Watery	Purple ring	+
14	Cyanogenic glycosides	Ethanol/Methanol/Watery	No red colour change	-

+ = presence, - = absence, ppt. = precipitate

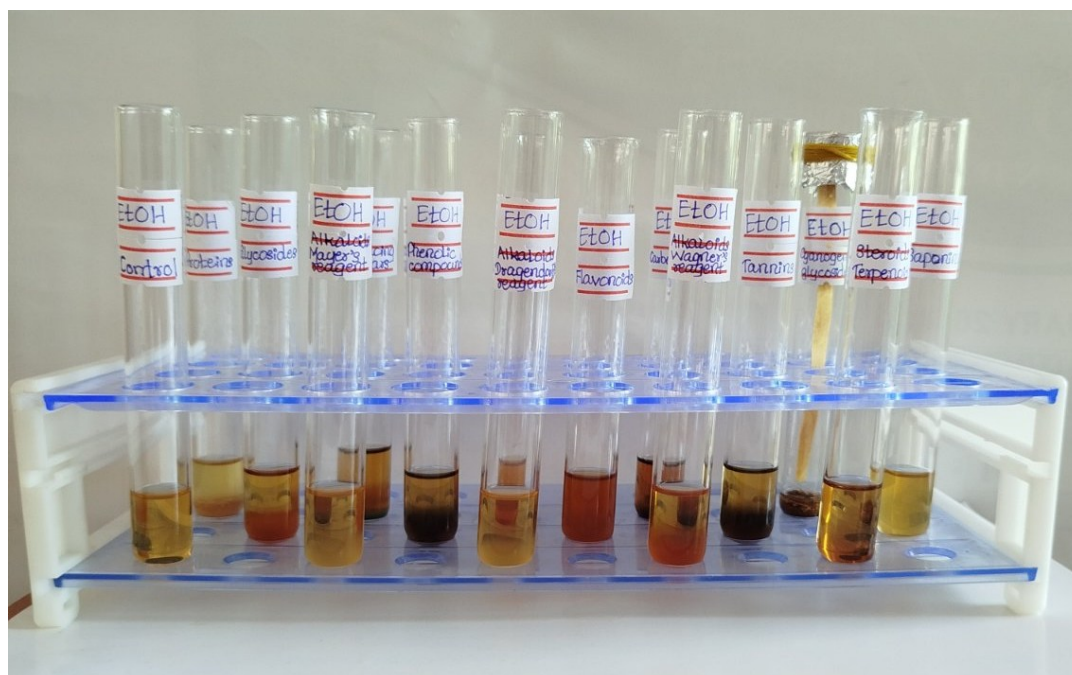


Figure 2. Phytochemical Analysis of Ethanolic Extract of Fruits of *A. subulatum* Roxb.

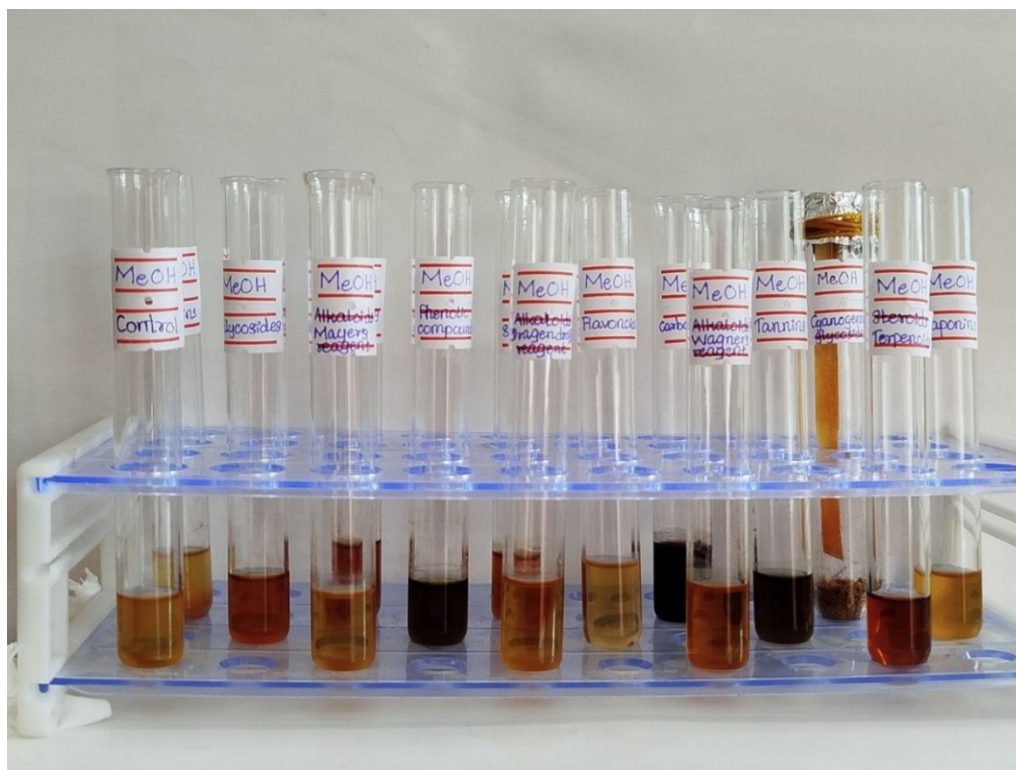


Figure 3. Phytochemical Analysis of Methanolic Extract of Fruits of *A. subulatum* Roxb.

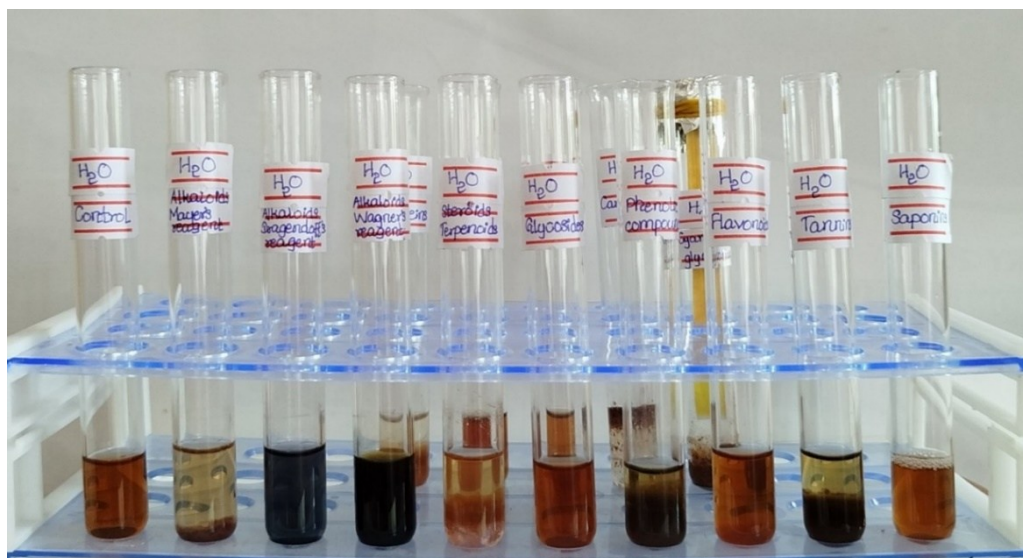


Figure 4. Phytochemical Analysis of Watery Extract of Fruits of *A. subulatum* Roxb.

Antioxidant activity of the fruit extracts of *Amomum subulatum* Roxb.

Antioxidant activity of dried powdered fruits extract was determined by DPPH screening assay that IC₅₀ value of watery extract was founded to be 11.9 ppm (11.9 µg/ml). Therefore, the dried powdered fruits extract of *Amomum subulatum* Roxb. had very strong antioxidant activity (Table 2, Table 3, Figure 5, Figure 6).

Table 2. Results of % Inhibition Vs Different Concentration of Ascorbic acid (mg/L)

Standard concentration (mg/L)	Absorbance	% Inhibition	IC ₅₀ (mg/L)
50	0.264	89.4146	6.75
25	0.724	70.9703	
12.5	1.123	54.9719	
6.25	1.39	44.2662	

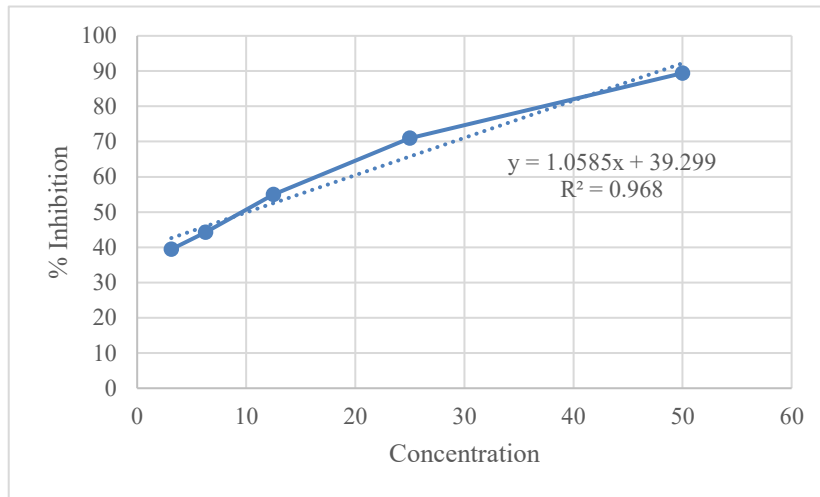


Figure 5. Plot of % Inhibition Vs Different Concentration of Ascorbic acid (mg/L)

Table 3. Results of % Inhibition Vs Different Concentration of Powdered fruits of *Amomum subulatum* Roxb. (mg/L)

Sample concentration (mg/L)	Absorbance	% Inhibition	IC ₅₀ (mg/L)
100	0.912	52.5	11.9
50	0.929	51.6146	
25	0.954	50.3125	
12.5	0.965	49.7396	

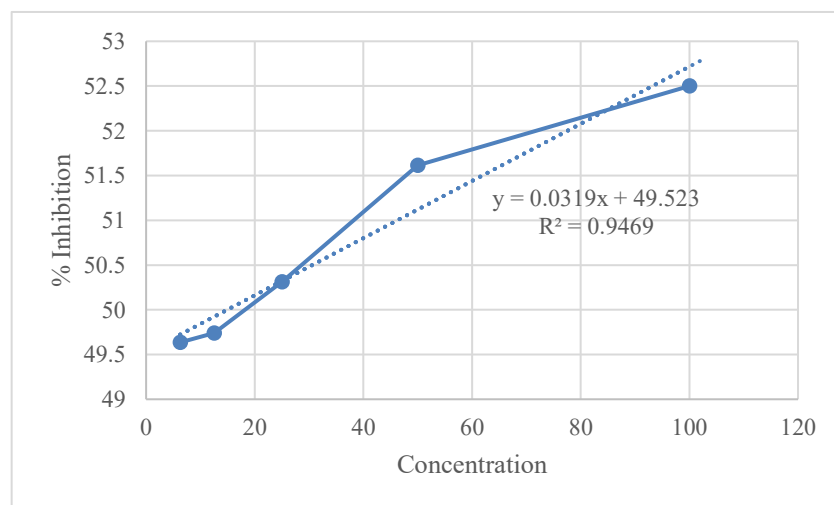


Figure 6. Plot of % Inhibition Vs Different Concentration of Powdered fruits of *Amomum subulatum* Roxb. (mg/L)

Nutritional Values of Fruits of *Amomum subulatum* Roxb.

Assessment of Nutritional Values were carried out at the U KAR KA Food Testing Laboratory, Chanmyatharsi Township, Mandalay Region. The results of nutritional values of the fruits of *Amomum subulatum* Roxb. showed that the highest contents of moisture, fiber and carbohydrate. The results were shown in Table 4 and Figure 7.

Table 4. Results of nutritional values of fruits of *Amomum subulatum* Roxb.

No.	Types of Nutrients	Contents %
1	Moisture	12.62
2	Total Ash	4.86
3	Crude Fat	1.14
4	Crude Fiber	15.45
5	Crude Protein	6.2
6	Carbohydrate	59.73

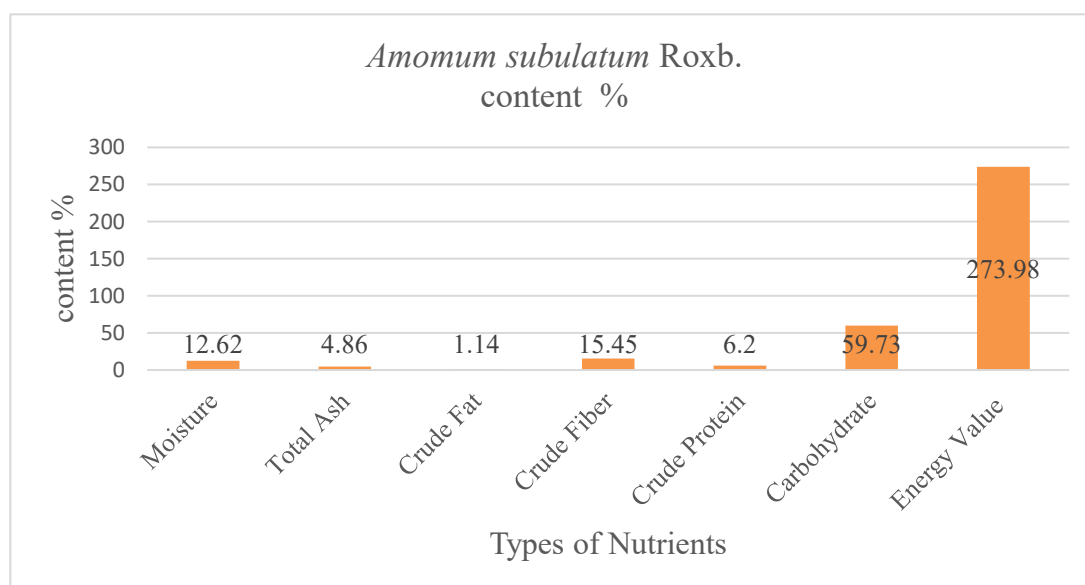


Figure 7. Showing the nutritional values of fruits of *Amomum subulatum* Roxb.

Discussion and Conclusion

In the present study, phytochemical properties, the assessment of nutritional values and antioxidant activity from fruits of *Amomum subulatum* Roxb. were investigated. Firstly, the phytochemical analysis revealed that the dried powdered fruits of *Amomum subulatum* Roxb. contained bioactive components such as alkaloids, steroids, terpenoids, glycosides, phenolic compounds, flavonoids, tannins, saponins, α -amino acids, proteins, reducing sugars, starch, and carbohydrates, consistent with previous studies. Mou *et al.* (2024) identified carbohydrates, alkaloids, glycosides, phenolic compounds, flavonoids, tannins, and steroids in the ethanolic extracts of the seeds. Similarly, Alam *et al.* (2020) reported the presence of glycosides, phenolic compounds, sterols, flavonoids, carbohydrates, tannins, volatile oils, proteins, and amino acids in the ethanolic extract of the fruit. Shivanand & Mahalaxmi (2010) observed carbohydrates, flavonoids, amino acids, steroids, triterpenoids, glycosides, tannins, and phenolic compounds in metabolic

extracts of the fruit. Additionally, Lakshmi *et al.* (2022) identified alkaloids, carbohydrates, flavonoids, saponins, tannins, glycosides, fixed oils, and fatty acids in the aqueous extract of *A. subulatum* Roxb. fruits.

The fruits of *A. subulatum* Roxb. contained bioactive compounds such as 1,8-cineole, α -terpineol, protocatechualdehyde, and protocatechuic acid, which exhibit antioxidant properties by inhibiting lipid peroxidation and offering significant health benefits. These active components are linked to the modulation of antioxidant enzymes in the liver and heart (Gautam *et al.* 2016). The fruits were a rich source of antioxidants, capable of neutralizing free radicals and mitigating oxidative stress, a key factor in degenerative diseases like diabetes and cancer. As a natural antioxidant source, *A. subulatum* Roxb. had gained popularity for its therapeutic potential (Singhal *et al.* 2022). Mehvish & Muhammad (2017) reported that the antioxidant activity of *A. subulatum* Roxb. reached 8.25 ± 2.0 $\mu\text{g/mL}$, demonstrating a high level of activity. Similarly, Alam & Majumdar (2018) evaluated the essential oil of three cultivars of *A. subulatum* Roxb. fruits and found antioxidant activity of 84.74%, 72.38%, and 64.42%. In the present study, the watery extract of dried powdered fruits of *A. subulatum* Roxb. showed very strong antioxidant activity with an IC_{50} value of 11.9 ppm (11.9 $\mu\text{g/ml}$), further confirming its potential as a natural antioxidant.

The nutritional analysis of the fruits of *A. subulatum* Roxb. revealed high levels of moisture, fiber, and carbohydrates. Carbohydrates serve as the primary source of energy for the body, fueling vital functions such as brain activity, kidney operation, heart muscle performance, and the central nervous system. They are an essential component of a balanced diet for both children and adults. Crude fiber plays a crucial role in promoting digestion and stimulating the growth of beneficial intestinal bacteria. Meanwhile, moisture content significantly influences the processability, shelf life, usability, and overall quality of various products, including pharmaceuticals, plastics, and foods (Townsend *et al.* 2023).

Collectively, the results of this research indicated that the dried powdered fruits of *A. subulatum* Roxb. had the potential phytochemical constituents, nutritional values and antioxidant activity. Therefore, it can be concluded that the fruits of *A. subulatum* Roxb. possessed the potential therapeutic properties despite the needs for further investigations such as quantitative analysis and *in vitro* and *in vivo* cell experiments. It was believed that this research might be able to partially participate in natural drug development and support the use of plant-based resources in the food industries.

Acknowledgements

First of all, we would like to acknowledge with deepest gratitude to Rector Dr Soe Myint Aye and Prorector Dr Phyu Phyu Myint, Sagaing University, for their permission to publish this research paper. We intend to declare our gratefulness to Dr Kalaya Lu, Professor and Head, Department of Botany, University of Mandalay. We would like to give my thanks Professor Dr Kyi Kyi Win, Head of the Department of Botany, Sagaing University, for her kind permission to carry out this research work and for supporting the necessary facilities. We sincerely thank Dr Aye Nann Han, Professor, Department of Botany, University of Mandalay, for her constant, intensive, brilliant and invaluable guidance and good advice throughout this work.

References

- Alam, K., Devender, P., Vandana, A. S. & Quaisul, H. 2020. Evaluation of anthelmintic activity of *Amomum subulatum* fruits extract. Lloyd Institute of Management and Technology (Pharm), India.
- Alam, A. & Majumdar, R. S. 2018. Antioxidant activity of essential oil of three cultivars of *Amomum subulatum* and standardization of high-performance thin layer chromatography (HPTLC) method for the estimation of 1,8-cineole. Department of Biotechnology, Sharda University, India.
- Backer, C.A & R.C.B. Van Den Brink. 1968. Flora of Java. Vol III. Rijksherbarium, leyden, Noordhaff. Groningen Company, Netherlands.

- Bisht, V. K., Negi, J. S., Bhandari, A. K. & Sundriyal, R. C. 2011. *Amomum subulatum* Roxb: Traditional, phytochemical and biological activities. Herbal Research and Development Institute, India.
- Dassanayake, M. D. 1983. A revised handbook to the Flora of Ceylon, Vol. IV, University of Peradeniya, Washington, DC. Amerind Publishing Co. Pvt. Ltd., New Delhi.
- Dhakal, R., Anjum, D., Ruksana, S. A., Gupta, Dipanneeta, D. G., Ravi, K. S., Prachurijya, D., Pankaj, B., Prasenjit, M., Sastry, G. N. & Jatin, K. 2022. Prophylactic and therapeutic potential of active phytoconstituents from *Amomum subulatum* Roxb.
- Gautam, N., Rewati, R. B., Bai, K. S. K. & Prakash, O. 2016. Technology, chemistry and bioactive properties of large cardamom (*Amomum subulatum* Roxb.) an overview, Faculty of Mathematics and natural science, University of Bergen, Norway.
- Harbone, J.B. 1998. Phytochemical Methods. A Guide to Modern Techniques of Plant Analysis. Chapman and Hall Ltd., London.
- Hooker, j. D. 1894. Flora of British India. Vol. VI. Reeve co., Ltd, The Oast House, Brook, NR. Ashford, Kent. London.
- Khan. A., Ahmad, M., Sultan, A., Khan, R., Raza, J., Albidin, S. Z. U., Khan, S., Zafar, M., Uddin, M. N. & Kazi, M. 2024. Herbal Spices as Food and Medicine: Microscopic Authentication of Commercial Herbal Spice. Department of Plant Sciences, Quaid-i-Azam University, Pakistan.
- Kress, W.J., A. D. Robert., F. Ellen & Yin Yin Kyi. 2003. "A Checklist of the Trees, Shrubs, Herbs, and Climbers of Myanmar". Department of Systematic Biology-Botany, National Museum of natural History, Washington, DC.
- Lakshmi, K., A. Sowmya., R. Satya., Durga, P., Shaheena, B., M. Sucharitha., Anantha, B. L. P. & Abdul, N. 2022. Comparative invitro antihelmintic activity of *Eleteria cardmom* and *Amomum subulatum* against pheritima posthuman. Sri Siddhartha Pharmacy College, India.
- Mehvish, S. & Muhammad, Q. B. 2017. phytochemical and antioxidant screening of *Amomum subulatum*, *Elettaria cardamomum*, *Embllica officinalis*, *Rosa damascene*, *Santalum album* and *Valeriana officinalis* and their effect on stomach, liver and heart. University of Agriculture, Faisalabad.
- Mou, S. Z., Asaduzzaman, M., Fahima, A., Monirul, I., Nazmul, H. Z, Toufiq, E. K. & Anisuzzman. 2024. Pharmacological investigation of anti-coagulant, anti-hyperglycaemic, and anti-hyperlipidemic potential of ethanol extract of *Amomum subulatum* seeds. Pharmacy discipline, Khulna University, Bangladesh.
- Satyaj, P., Noura, S. D., Brittany, L. K. & William, N. S. 2012. Chemical compositions and biological activities of *Amomum subulatum* essential oils from Nepal. Department of Chemistry, University of Alabama in Huntsville, USA.
- Shaikh, J. R. & M. K. Patil. 2020. Qualitative tests for preliminary phytochemical screening. Pharmacology and Toxicology, College of Veterinary, India.
- Sharma, O. P. & Bhat, T. K. 2009. DPPH Antioxidant Assay Revisited. Food Chemistry, 113, 1202-1205.
- Sharma, V., Neha, L. Vikas, H. & Manoj, B. 2016. *Amomum subulatum* seed extract exhibit antioxidant, cytotoxic and immune-suppressive effect. Department of Biotechnology, Thapar University, India.
- Shekhar, S. & Prasad, M. P. 2015. Evaluation of antioxidant activity determination in Jasminum species by DPPH method. Department of Microbiology, Tumkur University, India.
- Shivanand, P. & Mahalaxmi, R. 2010. Identification and determination of protocatechuic acid present in greater cardamom fruit extracts by HPTLC technique. Manipal University, India.
- Shrestha, R. L. 2017. "GC-MS analysis, antibacterial and antioxidant study of *Amomum subulatum* Roxb". Department of Chemistry, Amrit Campus, Tribhuvan University, Nepal.
- Singhal, P. K., Girendra, K. G., Ravi, K. & Gaurav, K. 2022. A review on *Amomum subulatum* and *Elettaria cardamomum* with their pharmacological activity. Sri Ram Group of Colleges, India.
- Townsend, J. R., Trevor, O. K., Tess, M. M., David, D. C., Adam, R. J. & Ralph, E. 2023. Foundational Nutrition: Implications for Human Health. Department of Nutrition, Food Studies, and Public Health, New York University, USA.
- Xue, J. & Yin, Y. 2024. Plant-based food: from nutritional value to health benefits. College of Food Science and Engineering, Yangzhou University, China.