# Determination of Nutritional Values and Evaluation of Cytotoxicity Bioassay of *Oroxylum indicum* (L.) Benth (Kyaung-sha) Fruits

**Aung Kyaw Min[1], Tin Aung Kyaw[2], War War May Zin[3], San San Aye[4], Cho Cho Than[5]**

## Abstract

This research focused on the investigation of phytochemical constituents, nutritional values, and cytotoxicity bioassay from *Oroxylum indicum* (L.) Benth (Kyaung-sha) fruits. The samples were collected from the Pathein Township, Ayeyarwady Region, and identified at Botany Department, Pathein University. The preliminary phytochemical investigation of the *O. indicum* fruits revealed that alkaloids, α-amino acids, carbohydrates, flavonoids, glycosides, phenolic compounds, reducing sugars, saponins, steroids, tannins, and terpenoids were present while cyanogenic glycosides and starch were absent. The determination of nutritional values was measured by AOAC methods. According to the results, 15.12 % of ash, 21.34 % of carbohydrate, 5.23 % of fat, 36.21 % of fiber, 23.16 % of moisture, and 2.63 % of protein were found in *O. indicum* fruits. The soluble matter contents in some organic solvents such as petroleum ether, ethyl acetate, ethanol, and water were observed to be 42.20, 43.70, 221.20, and 259.30 mg/g respectively by WHO standard method. The cytotoxicity of watery and ethanol extracts of *O. indicum* fruits was determined by brine shrimp (*Artemia salina*) cytotoxicity bioassay. According to the results, the tested samples have a cytotoxic effect with the $LD_{50}$ value of watery extract (630.55 µg/mL) and ethanol extract (70.14 µg/mL).

**Keywords:** *O. indicum* (L.) Benth, nutritional values, cytotoxicity bioassay

## Introduction

Plants contain a broad range of bioactive compounds such as lipids, carbohydrates, phenolics, terpenoids, carotenoids, anthocyanins, flavors, and fragrances. These plants restrain materials that can be utilized for useful purposes, which are originators for the synthesis of drugs. Plenty of research work has been carried out on a number of medicinal herbs as well as they have been initiated to have definite action on the respiratory, nervous, circulatory, digestive, and urinary organisms, sexual organs, skin, hearing, vision, and taste. Nutritional value refers to the contents of food and the impact of constituents on the body. Medicinal plants possess essential food components such as carbohydrates, proteins, and fats as well as a variety of physiologically active components, including minerals and phytochemicals, that show numerous physiological effects on humans (Petrovska, 2012). These components are important for the human body's requirements and they are used in different physiological, metabolic, and morphological activities (Chauhan *et al*., 2021 and Cheeke, 2009).

**Botanical Aspect of *Oroxylum indicum* (L.) Benth (Kyaung-sha)**

| | |
|---|---|
| Family | Bignoniaceae |
| Botanical name | *Oroxylum indicum* (L.) Benth |
| English name | midnight horror, Indian trumpet flower |
| Myanmar name | Kyaung sha |
| Part used | Fruits |

[1] U, Assistant Lecturer, Department of Chemistry, Pathein University
[2] U, Lecturer, Department of Chemistry, Pathein University
[3] Dr, Associate Professor, Department of Chemistry, Pathein University
[4] Dr, Professor, Department of Chemistry, University of Yangon
[5] Dr, Professor and Head, Department of Chemistry, Pathein University

**Figure 1. Photographs of *O. indicum* (L.) Benth (Kyaung-sha) Fruits**

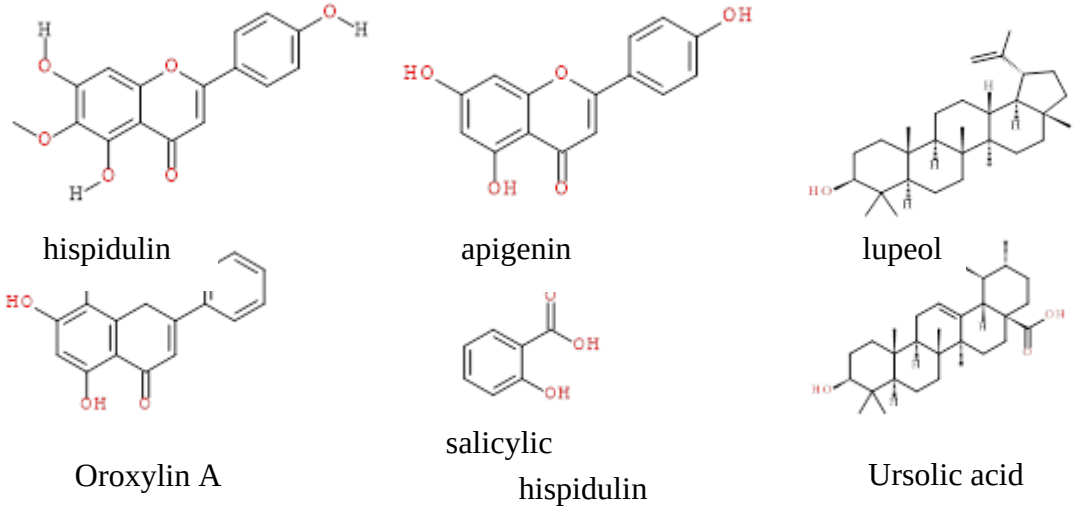


**Figure 2. Some reported chemical constituents of *O. indicum* (L.) Benth (Kyaung-sha) Fruits**

The main aim of this research was to investigate preliminary phytochemical constituents and to evaluate nutritional value and cytotoxicity bioassay from the fruits of *O. indicum* (L.) Benth (Kyaung-sha).

## Materials and Methods

Sample Collection

The fruit sample of *O. indicum* (L.) Benth (Kyaung-sha) was collected from Pathein Township, Ayeyawady Region in October 2020. The scientific name of the sample was identified by authorized botanists at Botany Department, at Pathein University.

Sample Preparation

The fresh sample was cleaned by washing with water and air-dried. The dried sample was ground using a grinding machine. And then this powdered sample was kept in a sealed air-tight container to prevent moisture changes and other contamination. It was then used without further purification or refining.

Preliminary Phytochemical Test

The dried powder of the selected sample 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 (Harborne, 1984).

Determination of Extractable Matter

Extractable matter determines the amounts of active constituents extracted with solvents from a given amount of medicinal plant material. Two extraction

methods are used for extractable matter contents such as the hot extraction method and the cold extraction method. The extractive values are useful to evaluate the chemical constituents present in the crude drug and help in the estimation of specific constituents soluble in a particular solvent. High alcohol soluble and water-soluble extractive values reveal the presence of polar substances like phenols, flavonoids, steroids, tannins, and glycosides. WHO method determines the number of active constituents extracted with solvents from a given amount of herbal medicinal plant materials. The extractive values provided an indication of the extract of polar, moderately polar, and non-polar components present in the herbal medicinal plant material (WHO, 1998).

Determination of Nutrient Parameters of *O. indicum* (L.) Benth (Kyaung-sha) Fruits

(i) Determination of Moisture Content

The moisture content of *O. indicum* (L.) Benth (Kyaung-sha) fruit sample was determined by oven-drying method (Egan, Kirk and Sawyer, 1981).

(ii) Determination of Ash Content

The ash content *O. indicum* (L.) Benth (Kyaung-sha) fruits sample was determined by the method given in "The Chemical Analysis of Foods" (Joslyn, 1970 and A.O.A.C, 2002).

(iii) Determination of Protein Content

Protein standard bovine serum albumin, copper sulfate pentahydrate, sodium potassium tartrate and sodium hydroxide from BDH were used for the determination of protein content (Roberts, and Elias 2011).

(iv) Determination of Fat Content

Fat content was determined by the Soxhlet extraction method using petroleum ether (Pearson, 1981).

(v) Determination of Total Carbohydrates Content

Phenol sulfuric acid method is the most reliable and easiest method among qualitative assay for carbohydrate estimation. The total carbohydrates content of the sample was determined UV-visible spectrophotometrically according to the phenol sulfuric acid method at the Department of Chemistry, Pathein University (Neeru,2014; Nielson, 2010 and).

(vi) Determination of Fiber Content

The fiber content was determined by the acid-base treatment method (Lee, 1975).

Determination of Cytotoxicity by Brine Shrimp Lethality Bioassay

In this experiment, the cytotoxicity activity of water and 70% ethanol extracts were investigated by brine shrimp lethality bioassay according to the procedure described by Dockery and Tomkins (2000).

## Results and Discussion

In order to classify the type of compounds present in plant samples, screening of phytoconstituents of *O. indicum* (L.) Benth (Kyaung-sha) fruit was performed by test tube method. According to these results, the selected plant generally contained alkaloids, α-amino acids, carbohydrates, flavonoids, glycosides, phenolic compounds, proteins, reducing sugars, saponins, steroids, terpenoids, and tannins. But cyanogenic glycosides and starch were found to be absent.

The extractable matter contents of *O. indicum* were determined by WHO standard methods. In order to know the extractable matter content of fruits of *O.*

*indicum* was determined in various solvent systems such as petroleum ether, ethyl acetate, ethanol, and water. However, water could extract more extractable matter content than other solvents. These results indicated that polar constituents were higher than non-polar. The extractable matter contents (mg/g) of *O. indicum* are shown in Table (1).

Table 1. Extractable Matter Contents of *O. indicum* (Kyaung sha) Fruit

| No. | Solvents | Weight of extractable matter (mg/g) |
|---|---|---|
| 1 | Petroleum ether | 42.2 |
| 2 | Ethyl Acetate | 43.7 |
| 3 | Ethanol | 221.2 |
| 4 | Water | 259.3 |

Nutritional value refers to the contents of food and the impact of constituents on the body. Medicinal plants possess essential food components such as carbohydrates, proteins, and fats as well as a variety of physiologically active components, including minerals and phytochemicals, that show numerous physiological effects on humans (Petrovska, 2012). These components are important for the human body's requirements and they are used in different physiological, metabolic, and morphological activities (Chauhan et al., 2021 and Cheeke, 2009).

In order to know the nutrient values of *O. indicum* amount of nutrients parameters such as protein, fat, fiber, carbohydrate, moisture, and ash were determined by appropriate methods. According to these observations, the fibers and moisture content of *O. indicum* samples were found to be the highest composition than other nutrients. The results are summarized in Table (2).

Table 2. Nutritional Values of *O. indicum* (Kyaung sha) Fruit

| **No.** | **Nutrient Parameter** | **Percentage of Content (%)** |
|---|---|---|
| 1 | Ash | 15.12 |
| 2 | Carbohydrates | 21.34 |
| 3 | Fat | 5.23 |
| 4 | Fiber | 36.21 |
| 5 | Moisture | 23.16 |
| 6 | Protein | 2.63 |

The cytotoxicity of watery and 70 % ethanol extracts of fruits of *O. indicum* was evaluated by brine shrimp cytotoxicity bioassay. The cytotoxicity of crude extracts was expressed in terms of mean ± SEM (standard error of mean) and $LD_{50}$ (50% Lethality dose). The resultant cytotoxicity of the sample at different doses (1000, 100, 10, and 1 μg/mL) is shown in (Table 3). This is useful in the means of toxicity testing because it is of high importance to determine the concentration range in which there is a linear correlation between the concentration and the lethality of the brine shrimps. In this experiment, standard potassium

dichromate ($K_2Cr_2O_7$) and caffeine were chosen because $K_2Cr_2O_7$ is well- known toxic agent used in this assay (Molina-Salinas and Said-Fermandez, 2006) and caffeine is a natural product. In evaluating herbal preparation for toxicity, the $LD_{50}$ values are commonly expressed either by comparison with Meyer's or Clarkson's toxicity index (Meyer et al., 1982; Lewis 1995 and Clarkson et al., 2004). The extracts with $LD_{50}$ values less than 1000 µg/mL are considered toxic, while extracts with LD50 greater than 1000 µg/mL are considered as non-toxic. Clarkson classified cytotoxicity as low toxic when the LD50 is between 500 and 1000 µg/mL, medium toxic when the $LD_{50}$ is between 100-500 µg/mL, while extracts with $LD_{50}$ of 0-100 µg/mL are highly toxic. According to these benchmarks, the $LD_{50}$ values of crude extracts of *O. indicum* varied between 70.14 – 630.55 µg/mL, these estimated that $LD_{50}$ values are lower than 1000 µg/mL.

Thus, based on the criterion of toxicity, these extracts has hight toxicity (Table 3). Studies have shown that there is a significant difference in the obtained $LD_{50}$ results for different crude extracts, mainly because some extracts are a poor medium for obtaining specific bioactive components (responsible for the toxicity) from this sample. The preliminary toxicity data obtained by conducting the brine shrimp lethality assay gives $LD_{50}$ values which are a convenient platform for further toxicity studies.

Table 3. Cytotoxicity of Watery and Ethanol Extract of *O. indicum* (Kyaung sha) Fruit

| **Samples** | **Dead % by using different concentrations (µg/mL) of samples** | | | | **$LD_{50}$** |
|---|---|---|---|---|---|
| | **1000** | **100** | **10** | **1** | |
| Watery | 66.70±0.58 | 26.70±0.58 | 6.70±0.58 | 0.00±0.00 | 630.55 |
| Ethanol | 80.00±1.00 | 63.20±0.58 | 23.30±0.58 | 10.00±0.00 | 70.14 |
| *Caffeine | 0±0 | 0±0 | 0±0 | 0±0 | >1000 |
| *$K_2Cr_2O_7$ | 100±0.10 | 74.67±0.11 | 73.13±0.47 | 48.63±0.19 | 1.50 |

*standard

## Conclusion

From the overall assessment of the present work concerning with determination of nutritional values and evaluation of cytotoxicity bioassay of *O. indicum* (Kyaung sha) fruits**,** the following inferences may be deduced.

From the preliminary phytochemical investigation, it was found that alkaloids, α-amino acids, carbohydrates, flavonoids, glycosides, organic acids, phenolic compounds, reducing sugars, saponins, steroids, tannins, and terpenoids were present in selected samples. Whereas, cyanogenic glycosides and starch were not detected in selected bitter Myanmar medicinal plants.

The extractable matter contents of the sample in the different polarities of solvents such as petroleum ether, ethyl acetate, ethanol and water were determined by using WHO standard method. It was observed that water could extract the highest

amount of constituents. It can be concluded that polar constituents were higher than non-polar.

The determination of nutritional values was measured by AOAC methods. According to the results, 15.12 % of ash, 21.34 % of carbohydrate, 5.23 % of fat, 36.21 % of fiber, 23.16 % of moisture, and 2.63 % of protein were found in *O. indicum* fruits. From these observations, the selected sample was rich in nutrients.

In the brine shrimp cytotoxicity bioassay, ethanol extracts have a high cytotoxic effect with the $LD_{50}$ value of 70.14 µg/mL indicating that the ethanol extract may have high potency of anticancer properties. The determination of these characteristics will aid future investigators in their pharmacological analyses of *O. indicum* (Kyaung sha) fruits**.**

The current study demonstrated that *O. indicum* (Kyaung sha) fruits are important medicinal plants. These possessed not only nutritional values but also processed cytotoxicity effects. So, this indicates that the selected sample may have high potency of anticancer properties.

**Acknowledgments**

I would like to thank the Department of Botany, Dagon University, for inviting me to present a research paper. I would like to express my profound gratitude to the Department of Higher Education (Lower Myanmar), Ministry of Education, Yangon, Myanmar, for the provision of the opportunity to do this research. I would like to express my gratitude to Rector and Pro-rectors Pathein University, for sharing their thoughtful ideas.

**References**

Ameh, S.J., O.O. Obodozie, U.S. Inyang, M.S. Abubakar and M. Garba. (2010). "Quality Control Testson Andrographis paniculata Nees (Family: Acanthaceae)– an Indian 'Wonder' Plant Grown in Nigeria", *Tropical Journal of Pharmaceutical Research*, **9** (4), 387-394

AOAC. (2002). "Official Method of Analysis". *International Food Research Journal*, **17**, 426-432

Badarinath, A.V., K.M.S.C. Rao, S. Ramkanth, T.V.S. Ranjan and K. Gnanaprakash. (2010). "A Review on In-vitro Antioxidant Methods: Comparisons, Correlations and Considerations", International Research Journal of Pharm. Tech, **2** (2), 1276-1285

Bele, A.A., and A. Khale. (2011). "Standardization of Herbal Drugs: An Overview", *International Research Journal of Pharmacy*, **2** (12), 56-60

Chauhan, P., Puri, S., Thakur, M., Rathour, S., Sharma, A. K., and Pundir, A. (2021). "A study of wild medicinal plants used in Nargu Wildlife Sanctuary of district Mandi in Himachal Pradesh, India". *Journal of Applied Pharmaceutical Science*, **11** (4), 135â-144

Dockery, M. and S. Tomkins, (2000). "Brine Shrimp Ecology". 1st Ed., The British Ecology Society, London, 92-93

Egan, H., R. S. Kirk., and R. Sawyer. (1981). *Pearson's Chemical Analysis of Food*, Churchill

Harborne, J. B. (1984). "Phytochemical Methods and A Guide to Modern Technique of Plant Analysis". London: Chapman and Hall, 37-222 Livingstone Inc., London, **123**

Kalu, I.G. (2010). "Identification and Traditional Uses of Some Common Medicinal Plants in Ezinihitte mbaise L.G.A., of Imo state, Nigeria", *International Research Journal of Pharmacy*, **2** (6), 1-8

Mkhombo, M.H. (2010). "The Effect of *Clausena anisata* (Willd) Hook [Rutaceae] Leaf Extracts on Selected Diabetic Related Carbohydrate Metabolizing Enzymes", School of Molecular and life Sciences at the University of Limpopo (Medunsa Campus), Research Longman Group Ltd., 453 *Paper*, 1-85

Petrovska, B. B. (2012). *Historical review of medicinal plants' usage*. Pharmacognosy reviews, 6 (11), 1.

Rekha, C. and M. Poornima. (2012). "Ascorbic Acid, Total Phenolic Content and Antioxidant Activity of Fresh Juices of Four Ripe and Unripe Citrus Fruits", *International Journal of Pharmaceutical Research*, **1** (2), 303-310

Samali, A. (2011). "Evaluation of Chemical Constituents of *Phyllanthus niruri*", *African Journal of Pharmacy and Pharmacology*, **6** (3), 125-128

Senha, J.A. and S. Chaudhari. (2011). "Alpha-Amylase Inhibitory and Hypoglycemic Activity of *Clerodendrone multiflorum* Linn. Stem", *Asian Journal of Pharmaceutical and Clinical Research*, **4**(2), 1-4

Nickavar, B. and L. Abolhasani. (2008). "Alpha-Amylase Inhibitory Activities of Six Salvia Species", *Journal of Pharmaceutical Research*, **7** (4), 297 -303

Ghosh, S., M. Ahire, A. Patil and A. Jabgunde. (2011). "Antidiabetic Activity of *Gnidia glauca* and *Dioscorea bulbifera:* Potent Amylase and Glucosidase Inhibitors", *Research Article* (1), 1-10

Neeru, A. (2014). Carbohydrates in different types of fruits. *Periodic Research*, **3** (2), 112-114

Nielson, S. S. (2010). *Phenol-Sulfuric Acid method for total carbohydrates*. Food Science Text Series. **4**, 47-53

Person's. (1981). *Chemical Analysis of Foods*. Churchill Livingstone Inc., London and New York; 29.

Roberts, R. and R. Elias, (2011). *Determination of Carbohydrates Using Phenol Sulphuric acid Method*. In: Food Analysis (4thEd). S. Nielson (ed.): Springer.

WHO (1998) "*Quality Control Method for Medicinal Plant Materials*, In; Determination of Extractable Matter and Determination of Bitterness Value" Geneva, 30, 38.

Zainol, M.K. and S. Yusof. (2003). "Antioxidative Activity and Total Phenolic Compounds of Leaf, Root, and Petiole of Four Accessions of *Centella asiatica* (L) Urban", *Food Chemistry*, **81**, 575-581