PRELIMINARY PHYTOCHEMICAL INVESTIGATION AND NUTRITIONAL VALUE OF THE SMALLEST FLOWERING PLANT, *WOLFFIA ARRHIZA* (L.) HORKEL EX WIMMER. IN INNMA AREA, PYAY DISTRICT

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Abstract

Wolffia arrhiza (L.) Horkel ex Wimmer. is the smallest flowering plant. It is known as in local name Ye-au (water egg) and its belonging to the family of Lemnaceae. It is situated in the natural lake, Innma area of Pyay District. The *Wolffia* occurs in the form of colonies that form bright green mats over the surface of water. The specimens were collected on April, 2012 and September, 2019. In this paper, preliminary phytochemical examination and nutritional value of this plant has been undertaken. Preliminary phytochemical examination showed the presence of alkaloid, glycoside, phenolic compound, flavonoid, steroid, tannin, saponin, α-amino acid, protein, reducing sugar, starch and carbohydrate. Nutritional value of the plant contains energy (230 kcal), proteins (27.12%) and carbohydrates (26.55%). *Wolffia arrhiza* (L.) Horkel ex Wimmer. provides rich in proteinaceous contents.

Keywords: *phytochemical, nutritional values*

Introduction

In the plant Kingdom, the smallest flowering plant is also aquatic. It is *Wolffia*, belonging to the family Lemnaceae (duckweeds), known as water meal or water egg which is tiny globular plants without roots. Duckweeds, monocotyledonous aquatic plants, are represented by 37 species. The 37 species are categorized into five well-defined genera (Appenroth *et al.*, 2013). The flowering plants range in size from 1 mm (*Wolffia* sp.) to over 100 m tall (*Eucalyptus regans*) (Groombridge, 1992). *Wolffia* is a genus of eleven species which include the smallest flowering plants on Earth (Royal Botanic Gardens, Kew 2020). Wayne and Thorne, 1984 stated that the plant bodies of *Wolffia arrihza* are 0.4-1.3 mm long and 0.2-1.0 mm wide; upper surface intensely green; floating on or partially below water surface. Determination of *Wolffia* species on morphological basis alone is very difficult and sometimes not reliable (Landolt, 1994). *Wolffia* generally floats on the surface of the water. The plants are distributed throughout the world, particularly in warm temperate and tropical regions. It is native to Europe, Africa, and parts of Asia, and it is present in other parts of the world as a naturalized species (United States Department of Agriculture, 2018). It is greatly reduced flowering plant, without leaves or stems. Some botanists refer to the plant body as a "frond" or "thallus," The community plants of Ye-au includes giant duck weeds (*Lemna* sp.) and water fern (*Azolla* sp.) in the pond.

In Thailand, Laos and India, *Wolffia* has been harvested for food for many generations. The plant contains about 35% protein as the same protein content as soy beans. Water-egg plants (*Wolffia*) are used as proteinaceous foods as in Wolffia-muffins, Wolffia-tomato sandwichs, gourmet dishes and delicious Wolffia-apple pie la mode in India, Thai and Laos. The plant is widely used as the fodder for fishes, water birds, other invertebrates, buffalos, cows and pigs (Hillman and Culley, 1978). Chantiratikul *et al*., 2010 stated that the cultivated species of *Wolffia* contained 29.61% of protein.

Wolffia is eaten by herbivorous fish as well as a variety of waterfowl. It is also used both as fodder for cattle and pigs, and as a fertilizer because of its high phosphorus and nitrogen

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accumulation, in Africa, India, and Southeast Asia (National Academy of Sciences 1976). Carbohydrate is one of the most essential for factors for survival of human being and use for energy. Protein and fat are regulated the hormone in body activities. Protein plays an essential constituent of the diet of energy. Fiber helps digestion by stimulating muscular activity in the digestive tract (FAO, 1994). Flora of North America Editorial Committee (2000) notes that duckweeds are known to have a very high productivity and nutrient value. *Wolffia arrhiza* (L.) Horkel ex Wimmer. is found in the natural lake of Innma Inn near the Yetashe village in Innma Area of Pyay District.

In this paper, the microphotograph, morphological characters and vegetative propagation were also studied. In addition, the preliminary phytochemical test can be known for the constituents of bioactive compound. And then, the nutritional value of the plants was conducted. The main objectives are to know the outstanding character of *Wolffia arrhiza* (L.) Horkel ex Wimmer., to investigate the phytochemical constituents and to evaluate the nutritional values of this plant.

Materials and Methods

Study Area

The study area of the present work is located in latitude 18˚ 31' 50" N and longitude 95˚ 21' 30" E. It is located in Thegon Township, Bago Region (West).

Collection and Identification

The materials used in this study were collected from the natural lake of Innma Inn near the Yetashe village in Innma Area of Pyay District. The lake was full of water condition in raining season and it become drought in dry season. The specimens were collected on April 2012 for nutritional value and September, 2019 for phytochemical investigation. After collection, the specimens were used for identification in the Department of Botany, Pyay University. The morphological characters were studied with fresh specimens. The microscopical characters of the plants were examined under light microscope and then classification was also described. The morphological character and nomenclature were done according to Backer (1968), Datta (1969) and Lawrence (1951).

Preparation of powdered samples

The collected specimens were washed with water to remove impurities. After washing, the specimen was air dried for 30 days and ground to get powder and stored in air tight container to prevent moisture changes and contaminations.

Preliminary phytochemical test from powdered of plant

Phytochemical investigation of the dried powdered of *Wolffia arrhizal* (L.) Horkel ex Wimmer. was tested qualitatively for the presence or absence of chemical constituents namely alkaloid, glycoside, phenolic compound, flavonoid, steroid, tannin, saponin, α-amino acid, protein, reducing sugar, starch and carbohydrate. Preliminary phytochemical examination was carried out in the Department of Botany, University of Yangon according to the method of Central Council for Research in Unani Medicine (1987) and Trease and Evans (2002). The results were shown in figure (4) and table (1).

Examining the Nutritional Value of powdered specimens

Nutritional value of powdered specimens such as protein, crude fat and carbohydrate were carried out in Food Industries Development Supporting Laboratory (FIDSL), Lanmadaw

Township, Yangon. The powdered samples have been determined according to the procedures given in the methods of Association of Official Analytical Chemists (AOAC) Horwitz (1980). The results were shown in Figure (5 and 6).

Results

Morphological Characters of water egg plant, *Wolffia arrhiza* (L.) Horkel ex Wimmer.

Floating or submerged perennial herbs, without roots, the plant body reduced to a small or minute oval frond, oblong flat or globosely thallus, free floating, the largest frond 1.2 mm long and 0.5mm broad, the smallest frond 0.6 mm long and 0.275 mm broad; daughter segment or bud grow from retroverted pocket of the mother frond; colonial and primarily reproduces vegetative propagated; the flowers are not obtained in these collection Figure (1, 2 and 3).

Figure 1 Study area and collecting the water egg plants in Innma Inn

Figure 2 Micrograph of *Wolffia arrhiza* (L.) Horkel ex Wimmer. and selling the plants in the market

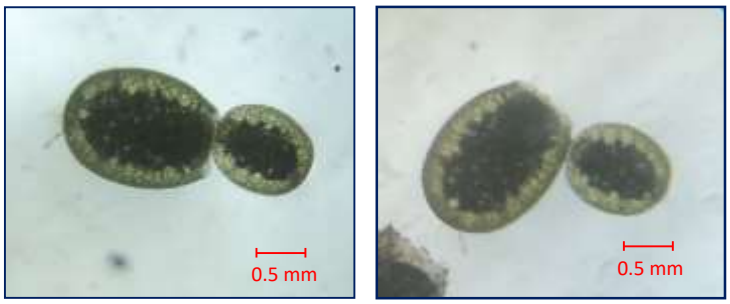


Figure 3 Vegetative Propagation showing budding stages of *Wolffia arrhiza* (L.) Horkel ex Wimmer.

Phytochemical Investigation of *Wolffia arrhiza* (L.) Horkel ex Wimmer.

The investigation of these test observed that the presence or absence of alkaloid, glycoside, phenolic compound, flavonoid, steroid, tannin, saponin, α-amino acid, protein, reducing sugar, starch and carbohydrate were shown in Figure (4) and Table (1).

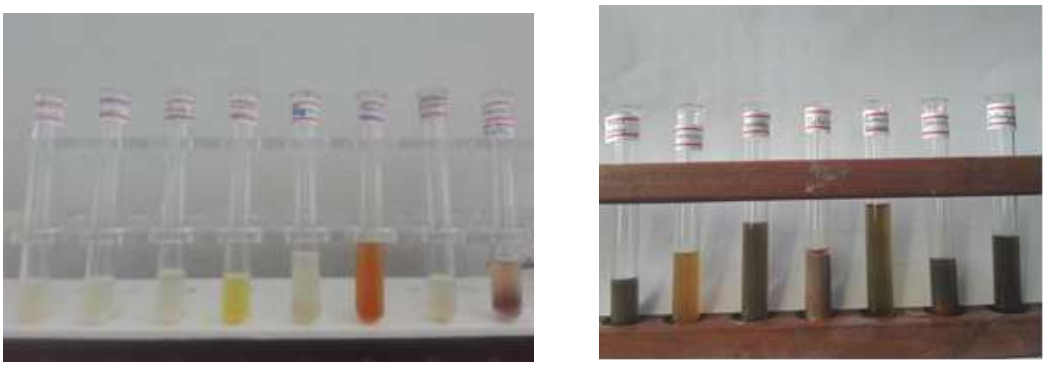


Figure 4 Phytochemical constituents of *Wolffia arrhiza* (L.) Horkel ex Wimmer.

Table 1 Preliminary Phytochemical investigation of *Wolffia arrhiza* (L.) Horkel ex Wimmer.

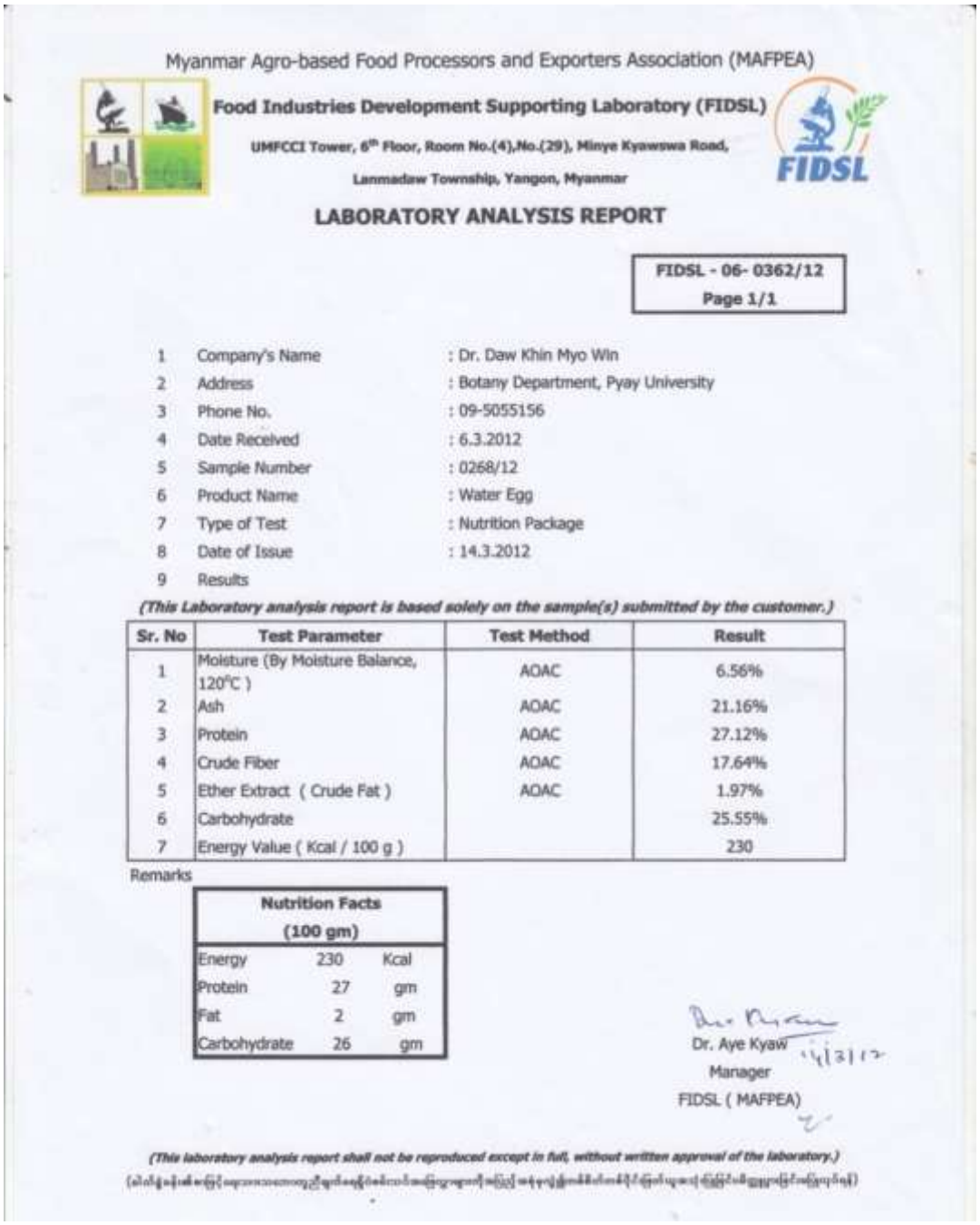
No.	Type of compounds	Extract	Test reagents	Observation	Results
1.	Alkaloid	Methanol	1%HCL+Mayer's reagent	White ppt.	+
		Methanol	1%HCL+Wagner's reagent	Reddish brown ppt.	+
		Methanol	1%HCL+Hager's reagent	Yellowish ppt.	+
2.	Glycoside	Methanol	1ml H_2O + NaOH	Yellow green color	+
3.	Phenolic compound	Methanol	2ml H_2O +10%FeC l_3	Green color	+
4.	Flavonoids	Methanol	Mg coil + HCl(dil.)	Reddish brown colour	+
5.	Steroid	Methanol	CH Cl_3 + H2So_4(conc.)	Green colour	+
6.	Tannin	Water	5%$FeCl_3$ + H_2So_4(dil.)	Yellowish brown ppt.	+
7.	Saponin	Water	Shaken with 2ml H_2O	Forthing	+
8.	α-amino acid	Water	Ninhydrin reagent	Pink spot	+
9.	Protein	Water	Millon's reagent(heated)	White ppt.	+
10.	Reducing sugar	Water	1ml H_2O and mixture equal part fehling's A and B	Brick red ppt.	+
11.	Starch	Water	Iodine	Brown ppt.	+
12.	Carbohydrate	Water	1 ml benedict's reagent and boil for few minute	Brick red ppt.	+

(+) = present ppts = precipitate

According to preliminary phytochemical test of *Wolffia arrhiza* (L.) Horkel ex Wimmer. presence of alkaloid, glycoside, phenolic compound, flavonoid, steroid, tannin, saponin, α-amino acid, protein, reducing sugar, starch and carbohydrate were observed.

Nutritional Value of *Wolffia arrhiza* (L.) Horkel ex Wimmer.

The experimental work for the nutritional value was carried out at the Food Industries Development Supporting Laboratory (FIDSL). According to the experiment, moisture, ash, protein, crude fiber, crude fat, carbohydrate and energy value were found. The results were shown in Figure (5 and 6).



Myanmar Agro-based Food Processors and Exporters Association (MAFPEA)

Food Industries Development Supporting Laboratory (FIDSL)

UMFCCI Tower, 6th Floor, Room No.(4),No.(29), Minye Kyawswa Road,
Lanmadaw Township, Yangon, Myanmar

FIDSL

LABORATORY ANALYSIS REPORT

FIDSL - 06- 0362/12
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1	Company's Name	: Dr. Daw Khin Myo Win
2	Address	: Botany Department, Pyay University
3	Phone No.	: 09-5055156
4	Date Received	: 6.3.2012
5	Sample Number	: 0268/12
6	Product Name	: Water Egg
7	Type of Test	: Nutrition Package
8	Date of Issue	: 14.3.2012
9	Results	

(This Laboratory analysis report is based solely on the sample(s) submitted by the customer.)

Sr. No	Test Parameter	Test Method	Result
1	Moisture (By Moisture Balance, 120°C)	AOAC	6.56%
2	Ash	AOAC	21.16%
3	Protein	AOAC	27.12%
4	Crude Fiber	AOAC	17.64%
5	Ether Extract (Crude Fat)	AOAC	1.97%
6	Carbohydrate		25.55%
7	Energy Value (Kcal / 100 g)		230

Remarks

Nutrition Facts (100 gm)		
Energy	230	Kcal
Protein	27	gm
Fat	2	gm
Carbohydrate	26	gm

Dr. Aye Kyaw
Manager
FIDSL (MAFPEA)

(This laboratory analysis report shall not be reproduced except in full, without written approval of the laboratory.)

Figure 5 Laboratory analysis report of nutritional package of water egg plants tested at Food Industries Development Supporting Laboratory (FIDSL) Yangon.

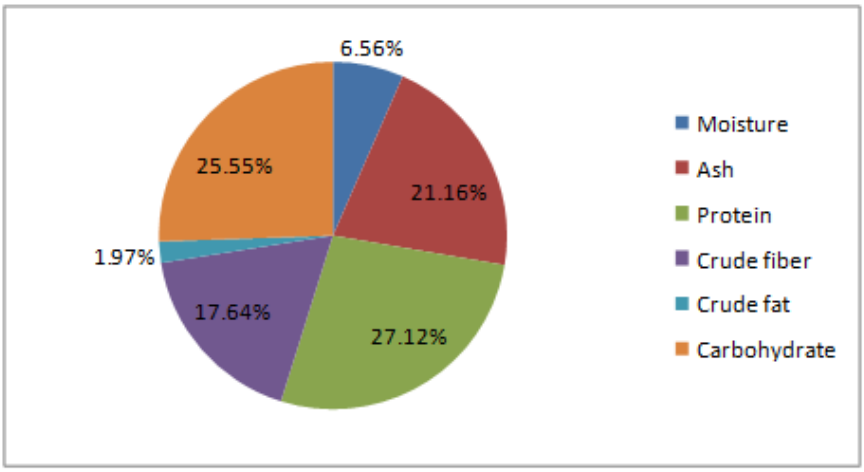

Figure 6 Nutritional contents of *Wolffia arrhiza* (L.) Horkel ex Wimmer.

According to this result, the content of protein, and carbohydrate were higher than the other; ash and crude fiber moderately presence; moisture and crude fat were found as small amount in *Wolffia arrhiza* (L.) Horkel ex Wimmer.

Discussion and Conclusion

In this paper, *Wolffia arrhiza* (L.) Horkel ex Wimmer. belonging to the family Lemnaceae. The plant were very small, free floating, perennial herbs, without roots, the plant body reduce to a small or minute, oval or oblong thallus which act as leaves. These characters were in agreement with those described by Backer (1968), Datta (1969) and Lawrence (1951). *Wolffia* is associated with giant duck weeds (*Lemna* sp.) and water fern (*Azolla* sp.) in the natural lake of Innma Inn. In this investigation, the plant bodies of *Wolffia arrihza* are 0.6 - 1.2 mm long and 0.275 - 0.5mm wide; upper surface green; floating on water surface. These parameters were similar to those given by Wayne and Thorne (1984). In this present paper, the water egg plants is failed to get the flowers of this tiny aquatic plants because absent of Scanning Electron Micrograph (SEM) during collecting period. The vegetative propagation by budding was thoroughly studied and photomicrographs of the budding stages were taken and presented.

In this study, the preliminary phytochemical investigation of the methanol and aqueous extracts of the whole plant of *Wolffia arrhiza* (L.) Horkel ex Wimmer. showed the presence of alkaloid, glycoside, phenolic compound, flavonoid, steroid, tannin, saponin, α-amino acid, protein, reducing sugar, starch and carbohydrate.

One of the purposes of this research was to study the nutritional contents of water egg plant such as the content of protein, and carbohydrate were higher than the other; ash and crude fiber moderately presence; moisture and crude fat were found as small amount in the *Wolffia arrhiza* (L.) Horkel ex Wimmer. Chantiratikul *et al*., 2010 stated that the cultivated species of *Wolffia* contained 29.61% of protein. In this research, *Wolffia arrhiza* (L.) Horkel ex Wimmer. contains 27.12%. The constituent of proteins were in similar to those mentioned by Chantiratikul *et al*., 2010.

Water-egg plants (*Wolffia*) are used as proteinaceous foods as in Wolffia-muffins, Wolffia-tomato sandwichs, gourmet dishes and delicious Wolffia-apple pie la mode in India, Thai and Laos. The plant is widely used as the fodder for fishes, water birds, other invertebrates, buffalos, cows and pigs (Hillman and Culley, 1978).

According to phytochemical investigations and nutritional contents, the presence of protein and carbohydrate in this plant which can strongly used for food in our country. In Myanmar, especially Innma, Shwedaung and Pyay Township people collect these plants and eat as frying and delight foods. The taste somewhat likes a sweet cabbage.

In conclusion, they are well grown in neutral pH. Water egg plants are widely grown in the lakes of Innma area, Shwedaung area and Pyay Township in Myanmar. It would be substituted of high priced meat and some vegetables for the people. Therefore water egg plants should be consumed because of its protein content value. And the information of the water egg plants should be published in journals and let them know it can be eaten and it can be partially fulfilled the meats of protein in daily diet cheaply. It is important to educate the local people to conserve and maintain the natural products of *Wolffia arrhiza* (L.) Horkel ex Wimmer.

However, it is expected that the present paper will give the knowledge of phytochemical constituents and nutritional value for some information of the smallest flowering plants of the world which is located in Innma, Shwedaung and Pyay Township of Myanmar.

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References

Appenroth, K. J., N. Borisjuk and E. Lam. (2013). **Telling duckweed apart: Genotyping technologies for *Lemnaceae*.** Chin. *J. Appl. Environ. Biol*. vol. 19, pp. 1-10.

Backer, C.A and R.C. Bakhu. (1968). **Flora of Java,** Vol.III. Wolters-Nordhalf, N.VGroningen - The Netherlands.

Bhanthumnavin, K., and M.G McGarry. (1971)**. *Wolffia* sp. as a possible source of inexpensive protein.** Nature. pp. 232, 495.

Central Council for Research in Unani Medicine. (1987)**. Phytochemical Standards of Unani Formulations.** New Delhi, Ministry of Health and Family Welfare.

Chantiratikul, A., O. Chinrasri, P. Chantiratikul, A. Sangdee, U. Maneechote and C. Bunchasak. (2010). **Effect of replacement of protein from soybean meal with protein from *Wolffia* meal (*Wolffia globosa* (L). Wimm.)** on performance and egg production in laying hens. Int. J. Poult. Sci., 9: 283-287.

Datta, S.C. (1969). **A Handbook of Systematic Botany**. Asia Publishing House, Bombay, India.

F.A.O (1994). **Tropical roots and crops production, perspectives and future prospects**. United Nation.

Flora of North America Editorial Committee. (2000). **Flora of North America,** North of Mexico. Volume 22: Magnoliaphyta: Alismatidae, Arecidae, Commelinidae (in part), and Zingiberidae. Oxford Univ. Press. New York, N.Y. pp. 352.

Groombridge, B. (1992). **Global Biodiversity.** Status of the Earth's Living Recources. Chapman & Hall, Landon.

Hillman, W.S. and D.D.Jr. Culley. (1978). **The use of duckweed.** American Scientist. vol. 66, pp. 442-451.

Horwitz, W. (1980). **Official methods of analysis of association of official analytical chemist**. (13rd ed.). Washington DC: Benjiamin Franklin Station.

Landolt, E. (1994). **Taxonomy and ecology of the section *Wolffia* of the genus *Wolffia* (Lemnaceae).** Berichte des Geobotanischen Institutes der ETH, Stiftung Rübel 60, 137–151.

Lawrence, G.H.M. (1951). **Taxonomy of vascular plants**. 10th Ed The Machillan company, Newyork, Landon.

National Academy of Sciences (1976). **Making aquatic weeds useful**: Some perspectives for developing countries.

Royal Botanic Gardens, Kew (2020). "***Wolffia* Horkel ex Schleid**". Plants of the World Online.

Trease, G.E. and W.C. Evans. (2002). **Pharmacognosy**. 15st Ed. Bailliere Tindall, Used in Food, Drugs and Cosmetics. John Wiley & Sons, Inc. New York.

Wayne, A. P. and R. F. Thorne. (1984). **The genus *Wolffia* (Lemnaceae) in California**." *Madro-o* 31, 171–179.

United States Department of Agriculture (2018). ***"Wolffia arrhiza".*** Germplasm Resources Information Network (GRIN). Agricultural Research Service (ARS).