Morphological, Microscopical Characters and Antioxidant activity of Leaves of *Talinum fruticosum* (L.) Juss.

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Abstract

Talinum fruticosum (L.) Juss. possesses numerous medicinal values and this plant belongs to the family Talinaceae. This plant was collected from Dagon Botanical garden, Dagon University. The morphological and microscopical studies of the leaves were investigated. The plant was perennial herbs, mucilage present, and stem erect and succulent. The cells of both surface views were deeply wavy and stomata types were paracytic. The preliminary phytochemical tests were conducted from the powdered leaves. The presence of alkaloid, carbohydrates, glycosides, phenolic compounds, saponins, starch, terpenoids and steroid were mostly found in the examination. Antioxidant activity of leaves extracts were performed by using spectroscopic DPPH assay method. EtOH extracts of leaves showed more potent radical scavenging activity than those of aqueous extracts.

Keywords : paracytic, antioxidant

Introduction

Medicinal plants are the most important source of life saving drugs for the majority of the world`s population. Plants have been used for centuries as remedies for human disease. Herbs and plants used by traditional medicine practitioners contain a wide range of substances that can be used to treat chronic as well as infectious diseases. The medicinal value of these plants lies in some chemical substances that produce a definite physiological action in the human body. The most important of these bioactive compounds of plants are alkaloids, flavanoids, tannins and phenolic compounds.

The plant of *Talinum fruticosum* (L.) Juss. is commonly known as Cally-thaine in Myanmar and Waterleaf in English. *Talinum fruticosum* (L.) Juss. belongs to the family Portulacaceae. This family contains about 20 genera and 500 species. It is native to tropical America; grown in Tamil Nadu. The plant grows as a used in the weed along roadsides and cultivated as a pot plant throughout in Myanmar (Conqurist, 1981).

The waterleaf plant is an erect, glabrous, perennial herb, usually strongly branched. The roots are swollen and fleshy, the stems succulent, obtuse-angular to erect. The leaves alternate, simple, almost sessile and succulent. The flowers are bisexual, regular, pedicel long, recurring in fruits. Fruits are globose to ellipsoid capsule long, 3 valved, elastically dehiscent with many seeds. Seeds are compressed globose-reniform, tuberculate shinning black. Water leaf is eaten as vegetable throughout the tropics. The leaves have been implicated medically in the management of cardiovascular diseases like stroke and obesity. According to traditional medicine the leaves of waterleaf are used to treat polyuria, internal heat, measles, gastrointestinal disorders, hepatic ailments, cancer and diabetics (Swarna, 2013).

Talinum fruticosum (L.) Juss. has been also implicated medically in the management of cardiovascular diseases like stroke, obesity etc. It is used as softener of other vegetable species. It is consumed as a vegetable constituent of a sauce in Nigeria. It is widely distributed and consumed as a leafy vegetable in the Southern

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ecological ones. Its leaves are used as softener of other vegetable species in vegetable soup (Ezekwe *et al*., 2013).

Phytochemicals or phytonutrients from medicinal plants are plant products, plant foods, such as fruits, leaves, and seeds etc. which are easily available sources of antioxidants. Since time immemorial medicinal plants played a vital role in the cure and prevention of diseases. Researchers proposed that a diet rich in antioxidants from fruits, vegetables and leafy vegetables are associated with a lower risk of life threatening diseases and plant-based diet protects against oxidative stress related diseases. Vegetables and fruits contain high concentration of numerous redox-active antioxidants such as polyphenols, carotenoids, ascorbic acids and flavonoids which fight against hazardous oxidative damage of plant cells. Thus, the consumption of dietary antioxidants from vegetables and fruits is beneficial in preventing these diseases and it has been proven to substantially reduce the risk of cardiovascular diseases, cancers and neurodegenerative diseases, including Parkinson's and Alzheimer's diseases (Olajire *et al*., 2011 and Morrison *et al*., 2010).

Free radical formation is controlled naturally by several useful compounds known as antioxidants. Antioxidants protect the body from damaging oxidation reactions by reacting with free radicals. Antioxidants are proficient in stabilizing or deactivating free radicals before they attack cells (Percival, 1998). Epidemiological studies reported that many of antioxidant compounds owe anti-inflammatory, anti atherosclerotic, anti-tumor, anticarcinogenic, anti-bacterial and anti-viral activities in varied magnitude. However, the studies testify that intake of natural antioxidants reduce risk of cancer, cardiovascular diseases, diabetes and other diseases associated with aging, etc.

For these facts, in this research, morphological and microscopial characters of fresh specimens, preliminary phytochemcial tests and antioxidant activity of the dried powdered leaves of *Talinum fruticosum* (L.) Juss. were carried out. The aim of the present study was to identify the plant of *Talinum fruticosum* (L.) Juss. to examine the microscopical characters of leaves, to study phytochemical tests and to examine the antioxidant activity of the leaves.

Materials and Methods

Botanical Studies

The specimen of *Talinum fruticosum* (L.) Juss. were collected from Botanical garden, Department of Botany, Dagon University. After the collection, the fresh specimens were studied, measured in detail and recorded. Based on the resulting data the plants were identified with the help of literatures (Hooker,1875; Dassanayake,1996; Swarna,2013). Both the vegetative and reproductive parts of the fresh specimens were used for the morphological and microscopical characters studies.

For microscopical studies lamina, midrib and petiole were presented by using available literature in the department of Botany, by the method of Metcalf and Chalk, 1950; Esau, 1965 and Trease and Evans, 2002

The fresh specimens were examined by preparing freehand section and studied under microscope. Temporary mounts were prepared with glycerin. Powdered were also examined to get standardization for traditional medicine. The following chemical and reagent were used to examine for freehand sections and the powdered samples.

(i) Chloral- hydrate solution B.P for clearing reagent

(ii) Solution of phloroglucinol B.P followed by with concentrated hydrochloric acid for lignin.

(iii) Acetic acid and 80% sulphuric acid B.P for calcium oxalate crystals.

The samples were thoroughly washed with water and air dried in an open shaded area for about three weeks. The dry samples were pulverized by grinding machine into powder to study the powdered characteristic. The powders were cleared in chloral hydrate solution on a glass slide and observed under the compound microscope.

Chemical Studies

Preliminary Phytochemical examination of leaves of *Talinum fruticosum* (L.) Juss.

For preliminary phytochemical investigation, the collected plant parts were washed repeatedly with tap water and finally washed with distilled water. Then, they were shade dried and powdered with help of grinder and stored in air tight container for chemical analysis. Preliminary phytochemical examination of the leaves on *Talinum fruticosum* (L.) Juss. has been conducted with test reagent in Botany Department of Dagon University according to the methods described by Marini-Bettelo,1981; Trease & Evans,2002.

Elemental analysis of leaves of *Talinum fruticosum* (L.) Juss.

The relative concentration of elements was analyzed by using Energy Dispersive X-ray Fluorescence (EDXRF) spectrometer technique at the Universities Research Center, Yangon.

Test for Antioxidant Activity, Total Phenolic Content and Total Flavonoid Content of leaves of *Talinum fruticosum* (L.) Juss.

Extraction

Each powder sample 60g of *Talinum fruticosum* (L.) Juss. leaves were extracted using two solvents including 99% of ethanol and distilled water. The samples were soaked in ethanol for 12 hours and distilled water that was boiled in water bath (60˚C) for l hour. The two extracts were filtered through a sheet of filter paper (Whatman No. 1) and the filtrates were re-filtered through a 0.45 µm nylon membrane filter (GE Healthcare UK).The collected filtrated were dried in different processes. The ethanolic extract was concentrated using a rotary evaporator with water bath at (60˚C) and the aqueous extract was concentrated using a rotary evaporator with water bath at (80˚-90˚C). And then, the two extracts were dried by freeze drier at (-62˚ C). The antioxidant activity, total phenolic content and total falvonoid content were carried out at the Department of Oriental Herb Science, Iksan, Chonbuk University, Korea.

Test for Antioxidant Activity

Preparation of DPPH (1, 1-diphenyl-1-picrylhydrazyl)

In DPPH stock solution, 0.002 g of DPPH mixed with 50 ml of methanol. The solution was freshly prepared and stored in falcon tube wrapped with silver foil.

Preparation of Test sample solution

0.2g of test sample and 2 ml of methanol were thoroughly mixed by vortex mixer. Then, the mixture solutions were placed in centrifuge. After 10 minutes, the stock solutions were obtained.

Measurement of DPPH Radical Scavenging Activity by Spectrophotometeric method

The control solution was prepared by mixing 200 µl of methanol and 1.8 µl of DPPH. Similarly, the blank solution was prepared 2 µl of methanol only. The sample solution was prepared by mixing 40 µl of test sample solution, 160 µl of methanol and 1.8 µl of DPPH solution. All solutions were kept in the dark for 30 minutes. Then the absorbance of the solution was measured at 517 nm using a UV-1601 Shimadzu

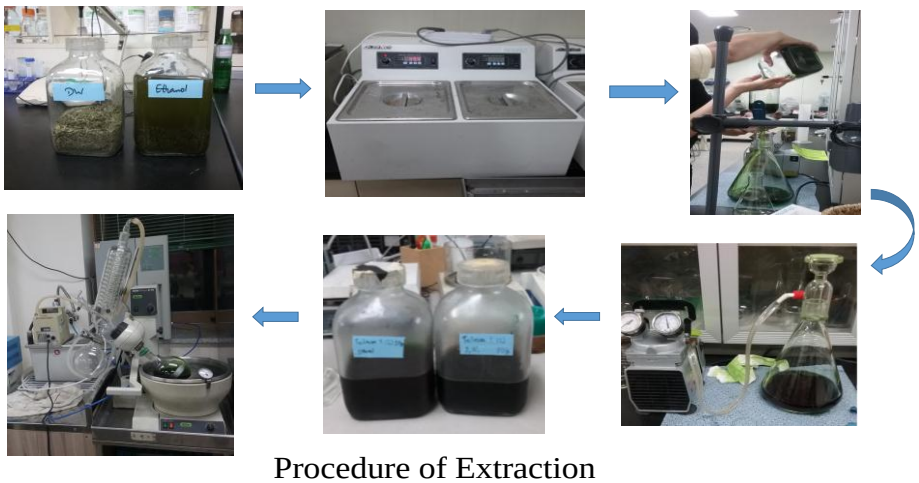
Spectrophotometer. These were performed in triplicate. The percentage inhibition was calculated using the following equation: %inhibition= (1- S/C) x100, S=Absorbance of sample, C= Absorbance of control. The results were shown in Table (2).

Total Phenolic Compound

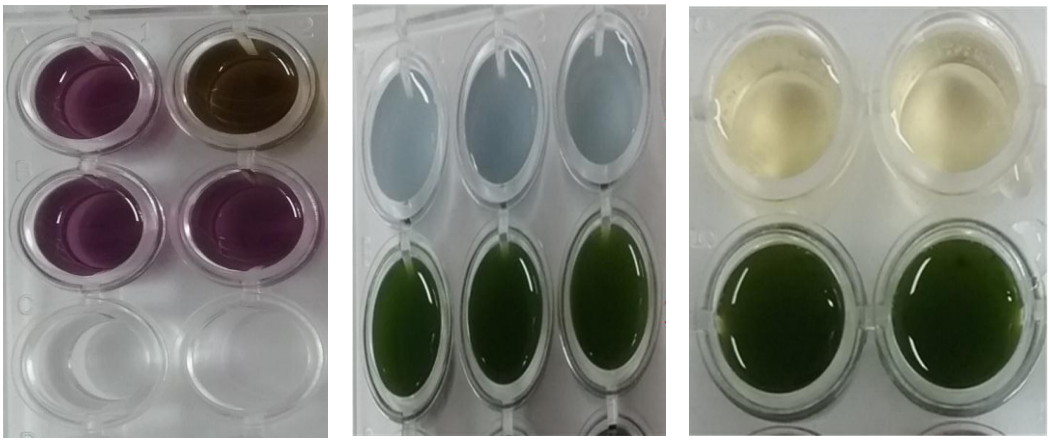
Total phenolic compound of each extract was determined by Folin-Ciocalteu's reagent. The sample 100 µl was prepared by mixing 2 ml of 2% Na_2CO_3. The mixture was left at room temperature for 3 minutes. Then, 100 µl of 50% Folin-Ciocalteu's reagent was added to the mixture and left for 30 minutes. The absorbance of the solution was determined at 700 nm using a UV-1601 Shimadzu Spectrophotometer. These were done in triplicate. The equivalent values of the extract were calculated using the following equation: Sample (Abs)-0.1523/0.8965. The results were shown in Table (2).

Total Flavonoid Content

Total Flavonoid content of each extract was determined by 10% $AlCl_3.6\ H_2O$. The sample 250 µl, 1ml of distilled water and 75 µl of 5% $NaNO_2$ were mixed and incubated for 5 mins. Then, 150 µl of 10% $AlCl_3.6\ H_2O$ was added into the mixture. After 6 minutes of incubation, 500 µl of 1M NaOH was added into the mixture and left for 11 minutes. The absorbance of the solution was determined at 500 nm using a UV-1601 Shimadzu Spectrophotometer. These were done in triplicate. The equivalent values of the extract were calculated using the following equation: Sample (Abs)-0.0848 / 0.0002.The results were shown in Table (2).



Procedure of Extraction



Test for Antioxidant Activity Test for phenolic compound Test for flavonoid content

Results

Botanical Studies

***Talinum fruticosum* (L.) Juss.**

Scientific name	-	*Talinum fruticosum* (L.) Juss.
Myanmar name	-	Cally-thaine
English name	-	Water leaf
Flowering Period	-	Almost throughout the year
Part used	-	Leaves and roots
Locality	-	Botany Department, Dagon University campus, East Dagon Township.

Perennial herbs, mucilaginous present. Stem erect, succulent, base pink, tip green, glabrous, roots swollen. Leaves simple, alternate, petiolate (very short), exstipulate. Inflorescences terminal and axillary; peduncles triangular, green, glabrous; Flowers bracteates, bracteolate, pedicellate, flattened, glabrous; complete, bisexual, regular, actinomorphic, pentamerous, cyclic, hypogynous. Sepals 2, aposepalous, broadly lanceolate, sepaloid (pale green), imbricate, inferior. Petals 5, apopetalous, petaloid (purple), imbricate, inferior. Stamens numerous, free, cream color, filaments filiform, free, unequal, pink, anther yellow, dithecous, dorsifixed, longitudinal dehiscence, inferior. Ovary superior, green, globsoe, monocarpellary, unilocular, with many ovules in the locule, free central placenta; style filiform, pink color, stigma trifid, pink, and disc absent. Fruits obglobose, pale green in immature, yellow in mature, glabrous. Seeds numerous, kidney, black. Roots tuberous (Fig. 1-8).

Microscopica Characters of leaves of *Talinum fruticosum* (L.) Juss.

Lamina

In the surface view, the epidermal cells of both surfaces were parenchymatous cells, thin walled and compactly arranged. Stomata were slightly present on the upper surface and abundant on the lower surface. They were paracytic type, guard cells were reniform in shape with numerous chloroplast (Figure 9-10).

In transverse section, the arrangement of the lamina tissue was dorsiventral. Both the upper and lower epidermises were covered with a thin layer of cuticle. The epidermal cells were made up of parenchymatous single layered. The upper epidermal cells were rectangular in shape and the lower epidermal cells were barrel shaped. The mesophyll layered composed of palisade and spongy parenchyma. The palisade mesophyll was made up of two layers of vertically elongated cylindrical cells at right angle to the surfaces, which were closely packed with one another compactly arranged. Solitary and druses crystals of calcium oxalate were present in mesophyll layered (Figure 11).

Midrib

In surface view, the epidermal cells of both surfaces were made up of thin walled parenchymatous cells. They were polygonal to rectangular in shape along the length of the midrib. The lower epidermal cells were similar to the epidermal cells. (Figure 12).

Figure (1) Habit

Figure (2) Leaf in various sizes

Figure (3) Inflorescence

Figure (4) Flowers

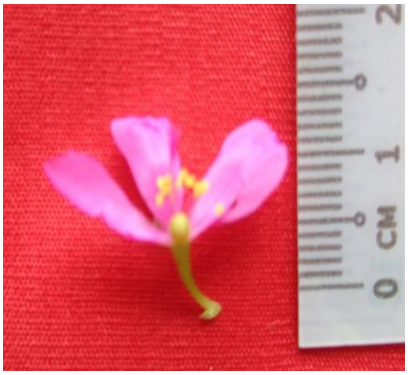


Figure (5) L.S of Flowers

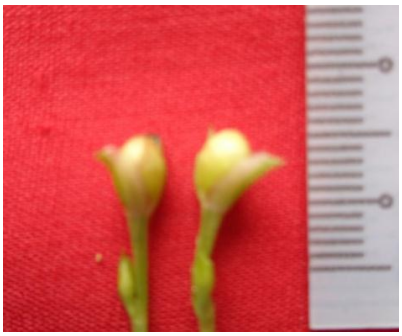


Figure (6) Fruits

Figure (7) Seeds

Figure (8) Roots

Morphological characters of *Talinum fruticosum* (L.) Juss.

In transverse section, the basal portion of the midrib was concave in the upper surface and convex in the lower surface. Both surfaces were covered with thick cuticle. Both epidermal cells were one layer, rectangular to round in shaped and compactly arranged. The lower epidermal cells were similar to those the upper epidermal cells. Below the epidermis, the cortex was made up of angular collenchymatous and thin walled parenchymatous cells. Intercellular spaces were numerous, solitary and druses crystals of calcium oxalate were present in both parenchymatous cells. The vascular bundles are crescent shaped in outline, collateral and closed type (Figure 13-14).

Petiole

In surface view, the epidermal cells of petiole were parenchymatous cells, thin walled and mostly rectangular in shape and elongated along the length of the petiole. Stomata are present (Figure 15).

In transverse section, the petiole was slightly 'v' shaped notch on the upper side and prominently rounded on the lower side. The cuticle layer was thick. The epidermal cells were rectangular or rounded in shaped. The cortex was made up of two different types of tissues below the epidermis, angular collenchymatous and parenchymatous tissues. Intercellular spaces were numerous among the tissues. Druses and solitary crystals of the calcium oxalate were present in the cells. The vascular bundles were crescent shape in outline and embedded in the parenchymatous tissues. Vascular bundles were arranged in collateral and surrounded by a bundle sheath (Figure 16-17).

Preliminary phytochemical test of leaves of *Talinum fruticosum* (L.) Juss.

The preliminary phytochemical test of the leaves of *Talinum triangulare* (Jacq.) Willd indicated the presence of alkaloid, carbohydrates, glycosides, phenolic compounds, saponins, starch, terpenoids and steroids; tannins, α-amino acids, flavonoids and reducing sugar are absence.

Table (1) Preliminary phytochemical test of leaves of *T. fruticosum* (L.) Juss.

No.	Test	Extract	Test Reagent	Observation	Result
1.	Alkaloids	1% Conc:HCl	1. Mayer's reagent 2. Dragendorff's reagent 3. Wagner's reagent	white ppt. yellow or red colour change	+ + +
2.	Carbohydrates	H_2O	10%α-napthol,Conc: H_2SO_4	red ring	+
3.	Glycosides	H_2O	10% lead acetate	white ppt.	+
4.	Phenolic compounds	H_2O	10% $FeCl_3$ & $K_3Fe(CN)_6$	deep blue ppt.	+
5.	Saponins	H_2O	Distilled water	frothing	+
6.	Tannins	H_2O	1%$FeCl_3$	brownish change	+
7.	Starch	H_2O	Iodine solution	blurish ppt	+
8.	α-amino acids	H_2O	Ninhydrin reagent	violet	+
9.	Reducing sugar	H_2O	Benedict's solution	pink colour	-
10.	Flavonoids	95%EtOH	Conc: HCl, Mg burning	light yellow	+
11.	Terpenoids	$CHCl_3$	Acetic anhydride, Conc: H_2SO_4	pink colour	+
12.	Steroids	Pet-ether	Acetic anhydride, Conc: H_2SO_4	pink colour	+

(-) = absent (+) = present (ppt.)= precipitate

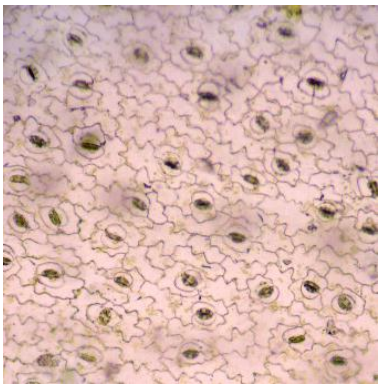


Fig. (9) Upper surface view of lamina (x 100)

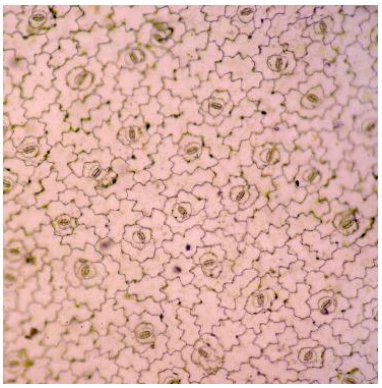


Fig.(10)Lower surface view of lamina (x 100)

Fig.(11) T.S of lamina (x 100)

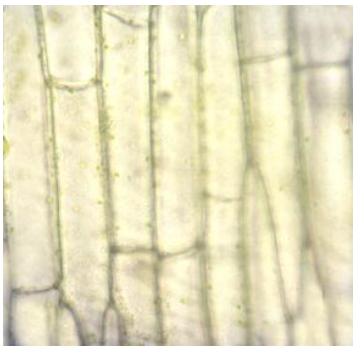


Fig.(12) surface view of midrib (x 100)

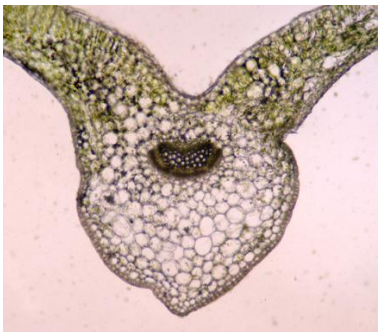


Fig.(13) T.S of midrib (x 40)

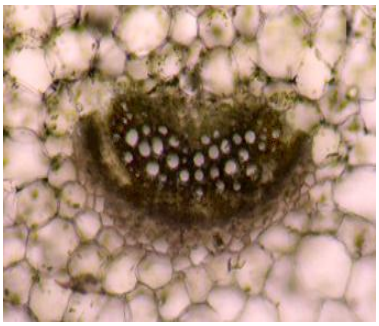


Fig.(14)T.S of midrib of vascular bundles (x 400)

Fig.(15) surface view of petiole (x 100)

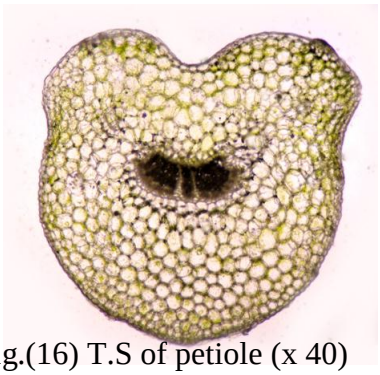


Fig.(16) T.S of petiole (x 40)

Fig.(17)T.S of petiole of vascular bundles (x 400)

Microscopical characters of leaves of *Talinum fruticosum* (L.) Juss.

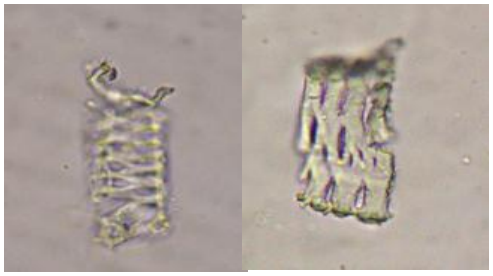


Fig.(18) Vessel (annular) and (pitted) (x 100)

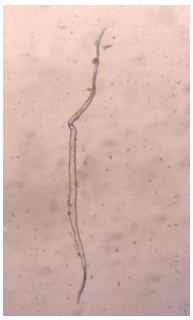
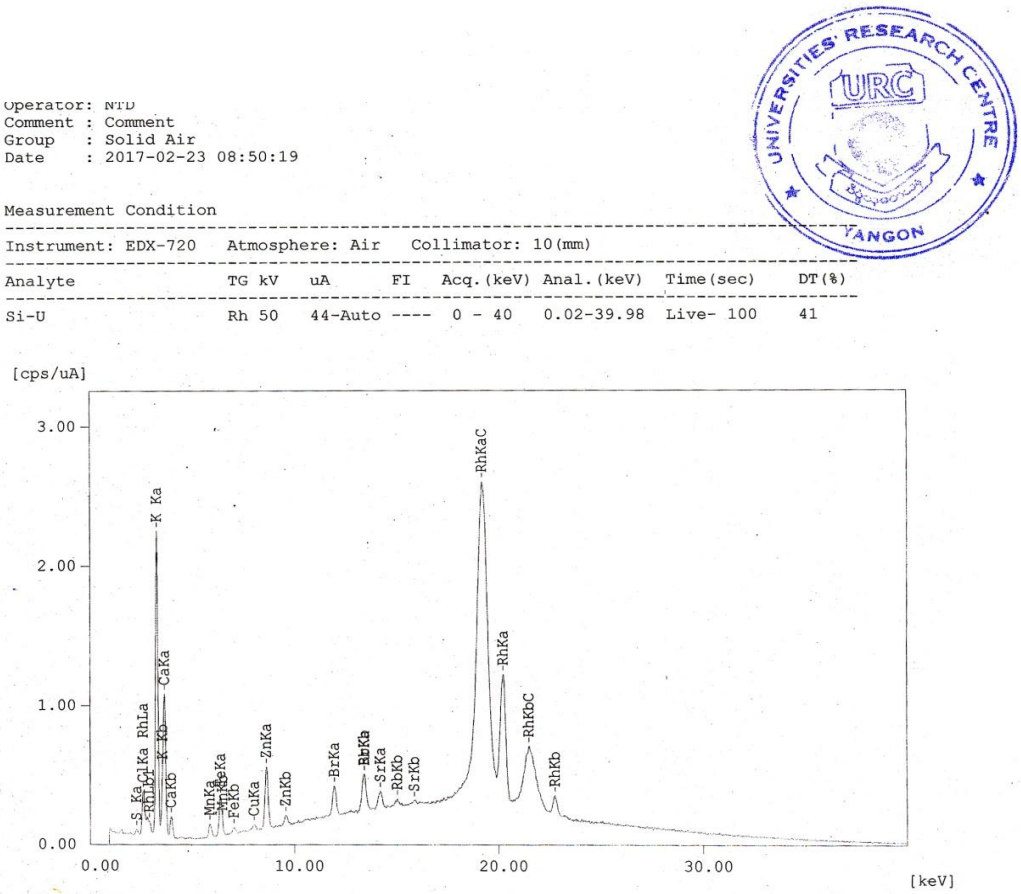


Fig.(19) Fiber (x 40)

Fig. (20) Tracheid (x 100)

Diagnostic Characters of Powdered leaves of *Talinum fruticosum* (L.) Juss.



Quantitative Result

Analyte	Result	[3-sigma]	Proc.-Calc.	Line	Int.(cps/uA)
K	51.781 %	[0.589]	Quan-FP	K Ka	14.9792
Ca	35.272 %	[0.521]	Quan-FP	CaKa	6.8240
Cl	5.983 %	[0.512]	Quan-FP	ClKa	1.1279
Fe	1.921 %	[0.047]	Quan-FP	FeKa	2.3484
Zn	1.584 %	[0.029]	Quan-FP	ZnKa	4.1809
S	1.299 %	[0.350]	Quan-FP	S Ka	0.2138
Mn	0.885 %	[0.083]	Quan-FP	MnKa	0.7984
Rb	0.446 %	[0.026]	Quan-FP	RbKa	2.9003
Br	0.443 %	[0.029]	Quan-FP	BrKa	2.2795
Sr	0.204 %	[0.021]	Quan-FP	SrKa	1.4864
Cu	0.183 %	[0.042]	Quan-FP	CuKa	0.3992

Figure (21) Relative concentration of elements contain in powdered leaves samples (%) by using ED-XRF

Elemental Analysis of *Talinum fruticosum* (L.) Juss. by using EDXRF

According to the EDXRF result, Potassium (K), Calcium (Ca), Chlorine (Cl), Iron (Fe), Zinc (Zn), Sulphur (S), Manganese (Mn), Rubidium (Rb), Bromine(Br), Strontium (Sr), Copper(Cu) were found in leaves of *Talinum triangualare* (Jacq.) Willd.

Test for Antioxidant Activity, Total Phenolic Compound and Total Flavonoid Content of leaves of *Talinum fruticosum* (L.) Juss.

According to this result, ethanolic extracts of leaves showed better scavenging activity than aqueous extracts.

Table(2) Antioxidant Activity of *Talinum fruticosum* (L.) Juss.

No.	Tests	Aqueous extract			EtOH extract		
		1	2	3	1	2	3
1.	DPPH Antioxidant activity	1.01%	5.95%	6.46%	28.09%	35.00%	37.74%
2.	Total Phenolic Compound	43.48	44.39	35.06	59.82	63.75	58.34
3.	Total Flavonoid Content	6.48	3.68	10.88	1566.08	1566.08	1566.08

Discussion and Conclusion

In this research, the morphological characters, microscopical characters, phytochemical and antioxidant activity of the leaves of *T. fruticosum* (L.) Juss. were presented.

In morphological study, *Talinum fruticosum* (L.) Juss. is a perennial herbs, mucilaginous present. The leaves are simple, alternate, petiolate, exstipulate, and margin entire. Inflorescences are axillary and terminal. Flowers are purple. Ovary superior, unilocular, many ovules in the locules, free, central placenta, stigma trifid, disc absent. Fruit is globose or ovoid, 3 valved. These are in agreement with Hooker 1875; Dassanayake, 1996 and Swarna, 2013.

In microscopical studies, the stomata are distributed on both surfaces of the leaves and paracytic types. The cell walls are wavy. Vascular bundles of midrib and petiole are crescent in shape. These characters are in agreement with Trease and Evean, 2002; Metcalf and Chalk ,1950 and Easu,1965.

In phytochemical studies, the leaves of *Talinum fruticosum* (L.) Juss. contained alkaloid, carbohydrates, glycosides, phenolic compounds, saponins, starch, terpenoids, and steroids. These characters are agreement with Swarna *et al*.,2013. Results of this study revealed that leaves contained an appreciable amount of flavonoids, alkaloids, saponins, among others and low level of toxicants like tannins, since it contains substantial amount of bioactive compounds.

According to Elemental Analysis, potassium (K) and calcium (Ca) were found as macronutrient elements. Potassium is found to be the highest percentage in leaves. Potassium is important for a healthy nervous system and a regular heart rhythm. It also regulates the transfer of nutrients to the cells. Calcium is vital in the formation of strong bones and teeth and is also important in the maintenance of regular heart beat and the transmission of nerve impulses (Dr Rob Hicks, 2015).

In antioxidant activity, *Talinum fruticosum* (L.) Juss. leaves extracts (ethanol and distilled water) were also studied for the free radical scavenging activity by using spectroscopic DPPH assay method. Aqueous and ethanolic extracts were prepared and

their free radical scavenging activities were evaluated. According to this result, EtOH extracts of leaves showed more potent radical scavenging activity than those of aqueous extracts. The study showed that *Talinum fruticosum* (L.) Juss. possesses considerable amounts of phenolic and flavonoid. The outcome of the total phenolic and flavonoid support the hypothesis that phenol rich plants are good sources of natural antioxidants which claim so many medicinal effects.

In conclusion, *Talinum fruticosum* (L.) Juss. is authenticated as a natural source of antioxidant in the form of a leafy vegetable. The above data would be helpful in further study of the plant parts and research and development in the field of medicine and can serve as a valuable resource in pharmaceutical and food industry. For the further research work, the bioactivity of this plant as anti-inflammatory, gestrointerstinal disorders and diabetic should be studied.

Acknowledgements

I would like to express my sincere gratitude to Professor Dr Myat Myat Moe, Head of the Botany Department, Dagon University, for providing all departmental facilities and valuable suggestions. I would like to express deepest gratitude to professor Keuk-Soo Bang, for support, expert guidance, immense knowledge throughout my study and research. I am also grateful to teacher Hyun-Gi, for helped some experiments. I want to indebted their sincere thanks to second Myanmar –Korea conference for their allowing submitting it this article

References

Conqurist (1981) **An Integrated System of Classification of Flowering Plants**, Columbia University, Press, New York.

Dassanayake, M.D.and W.D.Clayton. (1996). **A Revised Handbook to the Flora of Cyelon**, (Vol.X), Amerind Publishing Co.Pvt. Lid., New Delhi.

Dr. Rob Hicks (2015) **WebMD Medical**, Travel health Let's prepare for take off shop online at Boots. Com.

Easu (1965) **Plant Anatomy**, John Wiley & Sons INC, New York, London.

Hooker (1875) **Flora of British India** Vol. I, L. Reeve & Co. Ltd.

Metcalfe C.R and L. Chalk. (1950). **Amatomy of the Dicotyledons Leaves, Stem and Wood in Relation to Taxonomy with Notes on Economic Uses**, (Vol.I), Oxford, at the Clarend on Press.

Marini - Bettalo G.B., M.nicoletti and M. Patamia. (1981). **Plant Screening by chemical and Chromatographic procedure under field condition**. Journal of Chromatography, 31,14-17.

Morrison *et al*. (2010) **Comparative studies on the in *vitro* antioxidant properties of methano**lic & hydro-ethanolic leafy extracts from eight edible leafy vegetables of Ghana.

Olajire *et al*. (2011) **Total antioxidant activity, phenolic, flavonoid & ascorbic acid contents of Nigerian vegetables**. African Journal of Food Science & Technology.

Qi-ming (2007) **Flora of Hong Kong** Vol. I, Agriculture, fisheries & Conservation Department Government of the Hong Kong Special Administrative Region.

Percival (1998). M. Antioxidants. Clin. Nutr. insights, 1998, 1–4.).

Swarna *et al*. (2013) **International Journal of Pharmacy and Pharmaceutical Sciences** Vol. 5, Department of Plant Biology and Biotechnology. Loyola College.

Trease and Evean (2002) **Pharmacognosy**, 11th Ed, London.