Phytochemical Investigation on the Leaves of *Apium graveolens* L.

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Abstract

Apium graveolens L. (Tayok-nan-nan) was collected from Thingangyun Township, Yangon Region. Then the collected plants were classified, identified, verifying to confirm by the available literature. In morphological study *Apium graveolens* L. is annual aromatic herb, the leaves are tripinnately compound leaves, exstipulate. The inflorescences are axillary or terminal compound umbel, corolla obcordate and apices emarginated with inflexed tips, axile placentation. In microscopical study of *Apium graveolens* L. the lamina have wavy wall and anisocytic and anomocytic type of stomata. The mesophyll consists of palisade and spongy parenchyma cell. In transverse section of lamina, midrib and petiole, vascular bundles are oval to rounded shaped, collateral and closed type. In addition the dried powdered of leaves from *Apium graveolens* L. was also examined in this research.The qualitative and quantitative analysis were carried out to the standard chemical literatures. In qualitative analysis, the leaves of *Apium graveolens* L. showed that the alkaloid, glycoside, reducing sugar, saponin glycoside, carbohydrate, flavonoid, phenolic compound, steroid, α-amino acid, acid/base/ neutral, tannin and terpenoid were present. In quantitative analysis, leaf powder of this plant was found to be mostly soluble in water and moderately soluble in ethanol and methanol. In elemental composition of the this plant by using the Energy Dispersive X-ray Fluorescence (EDXRF), it was found that Calcium (Ca), Potassium (K), Chlorine (Cl), Iron (Fe), Copper (Cu), Zinc (Zn) Sulphur (S), Bromine (Br) and Stromium (Sr).The presence of toxic elements were analyzed by using the Atomic Absorption Spectophotometer (AAS), it was found Arsenic (Ar), Lead (Pb), Cadmium (Cd), Calcium (Ca),Iron (Fe), and Potassium (K).

Keywords: Morphological characters, Histological characters, Qualitative, Quantitative and Elemental composition of leaves of *Apium graveolens* L.

Introduction

Apium graveolens L. belongs to the family Apiaceae (Umbelliferae). This plant is commonly known as Toyok-nan-nan in Myanmar and celery in English (Hendley and Chit Ko Ko, 1967). The family contains about 300 genera and about 3000 species (Cronquist, 1981 and Heywood, 1978). This plant grows in marshes, ditches and other wet places and best on sandy and silt loam. It is abundant in temperate Europe, France, California, Florida, New York and Northern Asia (Kirtikar aand Basu 1935).

It is used as aphordisiac, anthelimintic, antispasmodic, emmenagogue, laxative, asthma, bronchitis, rheumatism, stimulant, Carminative, sedative, tonic and diuretic (Ashin Nargathein, 1973). Chinese use as treatment for high blood pressure. Leaves are used in soups, salads, sauces and diuretic for dropsy. This plant has been used widely both as a food and a medicine. Today, celery is a popular herb and vegetable in Europe, the leaves are sometimes chopped and used as a garnish, but more frequently cooked in soups and sauces to improve the taste. Celery leaves are used for spicing up foods and drinks such as cocktails.The leaves are used for therapeutic purpose in the preventing disease (Burkill, 1935).

The medicinal plants can be considered as biosynthetic laboratory of chemical compounds because there are many compounds like glycoside, alkaloids, terpenoids, phenols, resins and tannin etc. Those compounds responsible for therapeutic effects

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are the secondary metabolites. Plants are valuable source of natural drugs. In recent year, the scientists have been focused on medicinal plant research all over the world and use of herbal drugs had much increased in the few decades. Phytochemical progress has been aided enormously by the development of rapid ad accurate methods of screening plants for particular chemical. Classification of plant constituents are based on biosynthesis origin, solubility properties and the presence of certain key function group (Lister, 1995).

In this paper, qualitative analysis, quantitative analysis and elemental analysis from the leaves of *Apium graveolen* L. were conducted. The aim and objective of this paper is to investigate the chemical constituents of leaves of *Apium graveolens* L.

Materials and Methods

The specimens of *Apium graveolens* L. were collected from Thingangyun Township of Yangon Region. The specimens are verified with available literature such as Bailey 1939, Kirtikar and Basu 1933, Hooker 1885, Hooker 1879, lawrence 1951, Wealth of India 1966, 1948, Backer 1965, Trease & Evans 1978, Cronquist 1981, Satyanand Tyagi et al; 2013 .The leaves of the collected plants were thoroughly washed with water and then air dried in shade for about 2 weeks. These dried specimens were crushed and pounded into powder by using grinding machine, and stored in air tight container.

The following chemical and reagent were used to examine freehand sections and powdered samples. Solution of phloroglucinol B.P followed by concentrated hydrochloric acid for testing lignified wall. Chloral hydrate solution for clearing agent. Acetic acid B.P for testing Calcium Oxalate Crystal. Sudan III and IV solution for oil cells test.The qualitative and quantitative analysis were made according to British Pharmacopia (1973) and Trease and Evans, (2002). The analysis of elemental concentraction of *Apium graveolens* L. was carried out by using EDXRF and AAS spectrometer at University Research Center (URC).

Results

Morphological Characters

Family	-	Apiaceae
Botanical name	-	*Apium graveolens* L.
English name	-	Celery
Myanmar name	-	Tayok-nan-nan

Taxonomic Description

Annual aromatic herb, 20-60 cm in height, dichotomously branched from the base, stems angular, glabrous. Leaves alternate, tripinnately compound, 15.5-35.0 cm long, 4.5-10.0 cm wide, obtuse at the tip, the margin dentate, cuneate at the base, glabrous on both surface, petiole cylindrical, 13.5-20.0 cm long and 2.0-3.5 mm wide, exstipulate. Inflorescence terminal and axillary compound umbels, smaller and shorter than terminal ones, 2.3-3.1 cm long and 3.0-3.5 cm in diameter. Flower minute, white, 1.0-2.0 mm in length and 0.5 to 1.5 mm in diameter, bracts and bracteoles absent, pedicel 2.7-2.9 mm long, complete, bisexual, actinomorphic, pentamerous, epigynous. Sepals (5), fused, obsolete, valvate, glabrous; petals 5, free, obcordate with inflexed tips, nearly 0.5 mm length and in diameter, valvate, white in colour, glabrous; stamens 5, free, 1.0 mm long, alternate with petals, filament 0.1 mm in length and in diameter, slender, exerted, anther dithecous, dorsifixed, longitudinal dehiscence, bicarpellary,

bilocolar, bearing a single pendulous ovule in each locule, ovary ellipsoid or ovoid, 0.5-1.0 mm length and 0.9-1.1 mm in diameter, axile placentation, the style 2, the base forming a stylopod, stigma 2, simple; fruit schizocarpic, seeds 2 each mericarp one seeded.

Microscopical Characters of *Apium graveolens* L.

Lamina

In surface view, the epidermal cells of the lower surface was more wavy than the upper .The cells were polygonal in shape (Figs. 7 and 8).Stomata were present on both surfaces and more abundant on lower surface. The stomata of the upper surface was anisocytic and anomocytic of the lower.. The guard cells were reinform. Stomata index was 7-8-10 of upper and 8-10-12 of the lower. The trichome and glandular hairs were absent on both surfaces. Palisade ratio was 2-3-5 (Figs.5 and 6). Vein islets per square mm were 1-2-3 and vein terminations per square mm were 3-5-6.

In transverse section, the arrangement of lamina tissue is dorsiventral. The upper and lower epidermal cells were rectangular or barrel-shaped. The palisade mesophyll was made up of 1 to 2 layers of vertically elongated cylindrical cells, which are closed packed. They contain numerous chloroplast . The spongy mesophyll consisted of 4 to 6 layers of cells, which are irregular to isodiametric in shape and compactly arranged and measured (Fig. 9).The vascular bundle of lateral vein consisted of xylem the upper and lower of the phloem oval in shaped. This arrangement was collateral type. Vascular bundle was surrounded by a compact layer of thin-walled parenchymatous cells. The phloem tissue consists of sieve tube and companion cells. The phloem cells were very small. The xylem tissues consist of vessels, tracheid, fiber and xylem parenchyma cell The vessels were simple and pitted with simple perforation (Figs.1.42, 1.48). The fiber cells were long and the end walls were pointed.

Midrib

In surface view, the epidermal cells of both surfaces were parenchymatous and elongated along the length of the midrib. Trichomes and glandular hairs were absent on both surfaces. The stomata were present on both the surfaces. (Figs.11 and 12).

Transverse section of midrib was obcircular in outline (Fig.13). The upper surface of midrib was slightly convex and moderately concave in the lower. The cuticle layer of the lower surface was thicker than the upper. The lower cuticle was 2.7μ thick and 1.5μ in the upper. Both epidermal cells were polygonal and barrel shaped and measured. The cortex was made up of angular collenchyma and thin walled parenchyma cells. The collenchyma cells were 2 to 3 layers in thickness towards the upper and 1 to 2 layers toward the lower. They were rounded to isodiametric in shape and collenchyma cells. The parenchyma cells were 4 to 6 layers in thickness above the vascular bundle and 3 to 6 layers below. Vascular bundles were rounded to oval in outline, collateral type. Single vascular bundle was situated in the centre. The phloem tissue consisted of sieve tube, and companion cells. The xylem consisted of vessels, tracheids, fibres, and xylem parenchyma. The vessels have simple perforation and spiral thickening. The tracheids were annual, scalariform, and reticulate. The end wall is blunty acute. The fibre cells were long with tapering pointed (Fig.13).

Petiole

In surface view, the epidermal cells of the both surfaces were rectangular and elongated. The upper surfaces of stomata were anisocytic type .The lower surface of

stomata were anomocytic type. Trichome and glandular hair were absent (Figs. 14 and 15)

Transverse section of petiole was obcircular in outline. The upper surface of petiole was concave and straight or shallowly convex in lower. The lower cuticle thicker than the upper, the lower cuticle was 2.5μ thick and 2μ in upper. The upper and lower epidermal cells were barrel shaped and single layered. The cortex was made up of collenchyma and parenchyma cell. The collenchyma cells were 2 to 3 layers above and below vascular bundle the parenchyma cells were 4 to 8 layers above the vascular bundle and 3 to 5 layers below the bundle. They were rounded to isodiametric in shape.Vascular bundles were crescent shaped in outline, largest one located in the centre and other 6 bundles smaller toward the margin, collateral type (Figs. 16, 17). Phloem tissue consisted of sieve-tube elements and companion cells. The xylem consisted of vessels, tracheids, fibre, and xylem parenchyma. The vessels with simple perforation and spiral thickening .The tricheids were annual, reticulate, scalariform .The fibre cells were long with tapering pointed (Figs. 16 and 17).

Rachis

In surface view, the epidermal cells of both surfaces were elongated in shape. The upper surface of stomata was diacytic type .The lower surface of stomata were anisocytic type .The guard cells were reniform in shaped. The tirchomes were absent on both surfaces (Figs. 18 and 19).

Transverse section of rachis was oval in outline (Fig.20). Trichomes and stomata were absent. The upper cuticle was 5μ in thickness and 3μ at the lower. The lower and upper epidermal cells were barrel shaped and single layered. The cortex was made up of collenchyma and parenchyma cell. The collenchyma cells were 1 to 2 layers above and below vascular bundle .The parenchyma cells were 4 to 10 layers above and 4 to 6 layers below the vascular bundle. They were rounded to isodiametric in shaped. The vascular bundles were rounded to oval in outline, collateral type . The largest bundle was present in the centre and the lateral bundle decrease in size. Phloem tissue consisted of sieve-tube element and companion cells. Xylem consists of vessels, tracheids, and xylem parenchyma. The vessel is spiral thickening. The tracheids were helical, spiral and scalariform. The fibre cells were long, tapering pointed. Xylem parenchyma cells were irregular in shape (Fig. 20).

Morphological Characters of *Apium graveolens* L.

Fig. (1) Habit

Fig. (2) Leaves

Fig. (3) Infloresence

Fig. (4) Flower

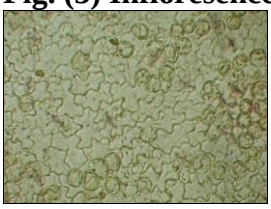

Fig. (5) Palisade ratio (X 100)

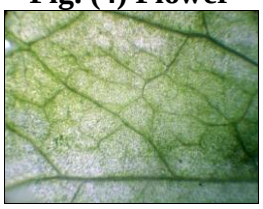

Fig. (6) Vein-islet and vein let Microscopical Characters of *Apium graveolens* L.

Lamina

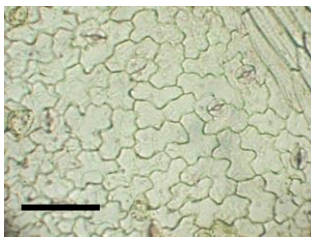

Fig. (7) Upper surface

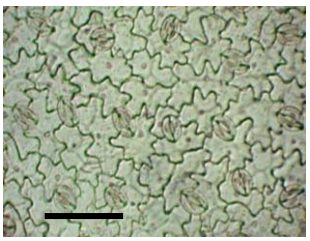

Fig. (8) Lower surface

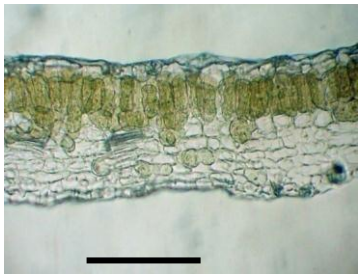

Fig. (9) T.S of Lamina

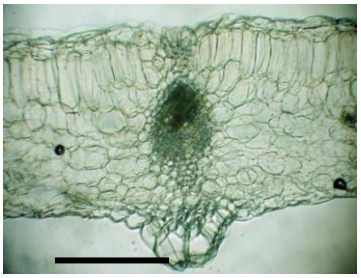

Fig. (10) Vascular bundle of lateral vein

Midrib

Fig. (11) Upper surface

Fig. (12) Lower surface

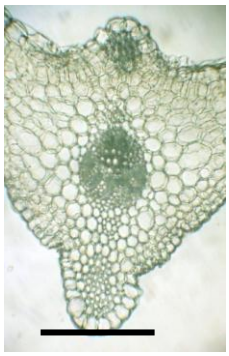

Fig. (13) T.S of midrib

Petiole

Fig. (14) Upper surface

Fig. (15) Lower surface

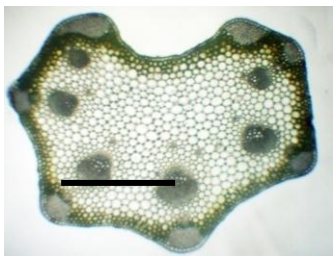

Fig. (16) T.S of petiole

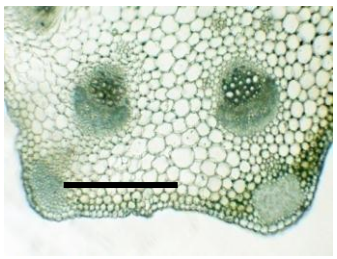

Fig. (17) T.S of petiole (basal portion)

Rachis

Fig. (18) Upper surface

Fig.(19) Lower surface

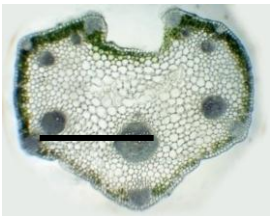

Fig. (20) T.S of rachis

Diagnostic Characters of Powdered Leaves of *Apium graveolens* L.

Fig. (21) Epidermal cells

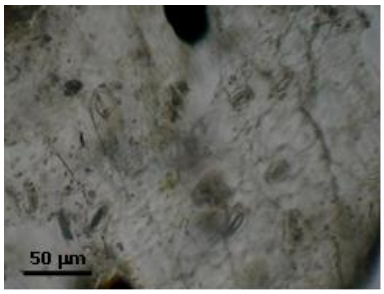

Fig. (22) Epidermal cells with stomata

Fig. (23)Vessel with scalariform thickening

Fig. (24) Vessel with spiral thickening

Fig. (25)Fiber

Qualitative Analysis

In Qualitative Analysis, alkaloid, glycoside, reducing sugar, saponin, carbohydrate, flavonoid, phenolic compound, steroid, α-amino acid, tanin and terpenoid were found to be present but cyanogenic glycoside was absent. The results were shown in Table 1.

Table 1.Qualitative analysis from the leaves of *Apium graveolens* L.

No.	Test	Extract	Test reagent	Observation	Result
1.	Alkaloid	3% HCl	1. Mayer's reagent 2. Dragendorff's reagent 3. Sodium picrate solution 4. Wanger's reagent	white ppt orange ppt yellow ppt yellow ppt	+ + + +
2.	Glycoside	H_2O	10% lead acetate solution	white ppt	+
3.	Reducing sugar	H_2O	1. Benedicts solution 2. Fehling solution	reddish ppt reddish ppt	+ +
4.	Saponin glycoside	H_2O	Distilled water	marked frothing	+
5.	Cyanogenic glycoside	H_2O	1. concentrated sulphuric acid 2. sodium picrate paper	no colour change	-
6.	Carbohydrate	H_2O	1. 10% α-naphthol 2. Conc: H_2SO_4	pink colour	+
7.	Flavonoid	Ethanol	C_6H_6	yellow colour	+
8.	Phenolic compound	H_2O	1% $FeCL_3$ solution	deep brown colour	+
9.	Steroid	Pet-ether	Acetic anhydride and conc: H_2SO_4	green colour	+

10.	α-amino acid	H_2O	Ninhydrin	pink colour	+
11.	Acid/base/ neutral	H_2O	Bromocresol green	Green	Neutral
12.	Tannin	H_2O	1. 3% Ferric chloride solution 2. Lead sub acetate solution	brown ppt	+
13.	Terpenoid	$CHCl_3$	1. Acetic anhydride 2. Conc: H_2SO_4	reddish brown	+

+ = Present , - = absent

Quantitative analysis of leaves of *Apium graveolens* L.

In quantitative analysis, the moisture content, total ash, acid insoluble ash and water soluble ash content were also determined and recorded. The extractive value of powdered leaves was investigated by using different solvents such as, ethanol, methanol, n-butanol, ethylacetate, chloroform, petroleum- ether and distilled water. The results were shown in (Table. 2).

Table 2. Quantitative analysis of leaves of *Apium graveolens* L.

No.	Physicochemical characters	Quality determined percent
1.	Moisture content	8.00
2.	Total ash	2.84
3.	Acid insoluble ash	0.91
4.	Water soluble ash	12.18
5.	Water soluble matter	10.57
6.	Ethanol soluble matter	5.56
7.	Methanol soluble matter	6.05
8.	n-butanol soluble matter	2.17
9.	Ethylacetate soluble matter	2.67
10.	Chloroform soluble matter	3.12
11.	Pet-ether soluble matter	2.00

According to the results, the solubility of powdered leaves in water was found to be the highest and moderately soluble in methanol and ethanol.

Elemental composition of *Apium graveolens* L.

The elements present in powdered leaves were quantitatively determined by EDXRF. It was found that Calcium (Ca), Potassium (k), Chlorine (Cl), Phosphorus (p) were found ass principle elements and Sulphur (S), Iron (Fe), Zinc (Zn), Copper (Cu), Manganese (Mn) and Stronium (Sr) as trace elements. The results were shown in (Fig.2 7) and (Table. 3).

Table 3. Elemental composition from the leaves of *Apium graveolens* L. by using EDXRF

No.	Elements	Concentration value (%)
1.	Ca	0.854
2.	K	1.049
3.	Cl	1.064
4.	Fe	0.021
5.	Zn	0.005
6.	Cu	0.004
7.	Sr	0.004
8.	S	0.345
9.	Br	0.003

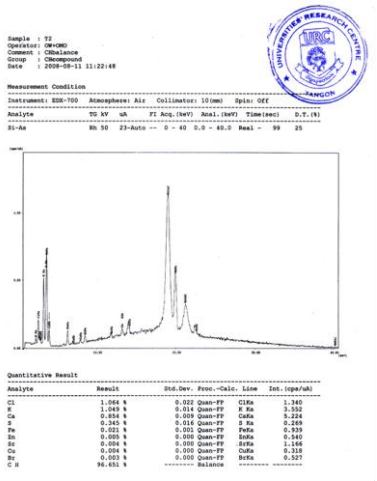

Fig. (27). (EDXRF) spectrum of the leaves of *Apium graveolens* L.

Atomic Absorption Spectrophotometric (AAS) analysis of leaves of *Apium graveolens* L.

According to the results of AAS, Arsenic (As) 0.024, Lead (Pb) 0.010, Cadmium (Cd), o.oo8, Calcium (Ca) 1.419, Iron (Fe) 0.058and Potassium (k) 0.007 were detected. The Atomic Absorption Spectrophotometric **(**AAS**)** analysis data of powdered leaves was given in (Table 4).

Table 4.Toxic elements analysed by using Atomic Absorption Spectrophotometer

Elements / Sample	As (ppm)	Pb	Cd (ppm)	Ca (ppm)	Fe (ppm)	K (ppm)
Apium geaveolens L. (leaves)	0.024	0.010	0.008	1.419	0.058	0.007

Discussion and Conclusion

In this research, morpholohical studied on both vegetative and reproduction parts of *Apium graveolens* L., the microscopical characters of leaves, qualitative analysis, quantitative analysis, the elemental composition were presented. *Apium graveolens* L. were collected from Thingangyun Township,Yangon Region. The flowering and fruiting periods are from December to March, respectively. This plants belong to the family Apiaceae .

As a result the plant of *Apium graveolens* L. Annual aromatic herb, dichotomously branched from the base, stems angular, glabrous. These characters described by Kirtikar and Basu 1935, Kihsherbarium Leydan 1948-1954, Lawrance 1951, Hooker 1879, Heywood 1978, Cronquist 1981.The leaves were alternate, tripinnately compound, obtuse of the tip, the margin dentate, cuneate at the base, glabrous on the both surface, petiole cylindrical, exstipulate. These characters are arranged with those given by Kirtikar and Basu 1935, Lawrance 1951, Rendle 1952, Backer 1965, Heywood 1978, Cronquist 1981.Inflorescence terminal and axillary, compound umbels. These characters in agreement with those described by Kirtikar and Basu 1935, Lawrance 1951, Hooker 1879, Cronquist 1981, Pandy 2000. Flower minute, white, regular ebracteate, ebracteolate, pedicellate, epigynous. These characters reported by Kirtikar and Basu 1935, Hooker 1879, Lawrance 1951, Backer 1965.The calyx fused, obsolete, glabrous. Corolla free, obcordate with inflexed tips, white, glabrous. These characters described by Kirtikar and Basu 1935, Kihsherbarium Leydan 1948-1954, Lawrance 1951, Backer 1965.The stamens 5, free,

alternate with petals, filament slender, exerted, anther dithecous, dorsifixed. The carpels 2, bilocular, bearing a single pendulous ovule in each locule, ovary ellipsoid or ovoid, axile placentation, style 2, the base forming a stylopod, stigma 2, simple. These characters in agreement with those described by Kirtikar and Basu 1935, Hooker 1879, Lawrance 1951, Cronquist 1981.The fruit schizocarpous. The seed glabrous, yellowish brown, endospermic. These characters reported by Hooker 1879, Rendle 1952, Backer 1965, Trease and Evan 2002.

In the present study, the microscopial characters of leaves were dorsiventrally and stomata were present on both surfaces with anisocytic type and anomocytic respectively. The epidermal cells were rectangular or barrel shaped the palisade and spongy parenchyma was presented in mesophyll. The vascular bundle of lateral vein was collateral type and distinctly parenchymatous bundle sheath. Trichome and glandular hair were absent. These characters were mentioned in Metaclaf and Chalk (1950) and Cornquist 1981, Pandey 2000. The surface view of rachis stomata were both surfaces diacytic type and anisocytic type respectively.

In transverse section of midrib, petiole and rachis the upper and lower epidermal cells were rectangular shaped. The cortex was made up of angular collenchyma and thin walled parenchyma cell. Vascular bundle was rounded to oval shaped, collateral type. Trichome and glandular hair were absent. These characters were described by Esau 1965, Eames and Lawrence 1974, Dutta and Mukerji (1952); and Metaclaf and Chalk 1950, Trease and Evan (2002).

In qualitative analysis, the leaves of *Apium graveolens* L. contained alkaloids, glycosides, reducing sugar, saponin glycoside, carbohydrate, flavonoid, phenolic compound, steroid, α-amino acid, acid/base/ neutral, tannin and terpenoid. Sofowara (1993) reported that celery is known to produce terpenoid, saponin, carbohydrate, flavonoids, alkaloids, steroids, glycosides, tannin and phenol. In quantitative analysis, seven different solvents were used. Powdered leaves were mostly soluble in water while they were moderately soluble in methanol and ethanol.

According to elemental analysis Calcium (Ca), Potassium (K),Sulphur (S) and Chlorine (Cl) were found macronutrient elements whereas Iron (Fe), Zinc (Zn), Copper (Cu) , Bromine (Br)and Strontium (Sr) were found as micronutrient elements. In this study calcium is found to be higher percentage in leaves..

The toxic elements such as arsenic, lead and cadmium were found as trace element in toxicity levels. The acute lethal dose of inorganic arsenic to humans has been estimated to be about 0.6 mg/kg/day (Mike, 1998).These toxic elements present in the leaves are less than harmful level, thus it could be used as traditional medicine. In conciusion, the result of present study will be useful for it proper effective utilization of Myanmar traditional medicine and further investigation.

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