Morphological, Microscopical Characters and Phytochemical tests of *Tithonia diversifolia* (Hemsl.) A. Grays

Yin Yin Khaing[1], Myat Myat Moe[2], Sann Sann Oo[3]

Abstract

It is a flowering plant known as *Tithonia diversifolia* (Hemsl.) A. Grays, belonging to the family Asteraceae. These plants wildly found in Loikaw Township were collected and identified. The morphological characters of vegetative and reproductive parts and microscopical characters of leaves were also studied. Morphology and microscopic examination of these plants were conducted to their correct identification. The plant was perennial robust shrubs and sparsely pubescent. Inflorescences were terminal, solitary head and involucre bracts presented. For microscopical studies, lamina, midrib and petiole were examined by preparing free hand sections. The epidermal cells of both surfaces of the lamina were slightly wavy. Stomata present on both surfaces were numerous and anomocytic type. The preliminary phytochemical tests were examined from the powdered inflorescences. Flavonoid, phenolic compound, tannin, saponin, amino acid and steroid were present in this examination.

Introduction

Medicinal plants are widely used in non-industrialized societies mainly because they are readily available and cheaper than modern medicine. In many countries, there is little regulation of traditional medicine but world health organization coordinates a network to encourage safe and rational usage. Medicinal plants face both general threats such as climate change and habitat destruction and the specific threat of over collection to meet market demand. Medicinal plants are important for pharmacological research and drug development not only when plant constituents are used directly as therapeutic agents but also when they are used as starting materials for the partial synthesis of drugs or as model for pharmacologically active compounds (WHO, 1998).

The Asteraceae are the largest family of vascular plants and the genera are estimated to number about 950 and the species probably 20000. They are distributed over most of the earth and in almost all habitats. The family is generally readily recognized by the inflorescence an involucrate head, the 5-lobed gamopetalous corollas, the usual presence of a pappus, the inferior bicarpellate uniloculate ovary with a single basal ovule and the 5 syngenesious stamens. The fruit an achene with a seed lacking endosperm is also distinctive (Lawrence,1964).

Tithonia diversifolia (Hemsl.) A. Grays is a species of flowering plant in the family Asteraceae that is commonly known as the tree marigold. It is native to Mexico and central America but has a nearly pantropical distribution as an introduced species. Depending on the area they may be either annual or perennial. It has shown great potentially raising the soil fertility in soils depleted in nutrients. This plant is weed that grows quickly and has become an option as an affordable alternative to expensive synthetic fertilizers. It has shown to increase plant yields and the soil nutrients of nitrogen (N), Phosphous (P) and potassium (K) (Website,1).

This plant was originally domesticated in Mexico and spread to other parts of Central and South America and North into the United States. It was brought over to parts of Africa and Asia as an ornamental plant and has become an invasive weed that is widely spread. It is most commonly found scattered among rivers and roadsides in

[1]Lecturer, Dr., Department of Botany, Dagon University
2 Professor and Head, Dr., Department of Botany, Dagon University
3Professor and Head, Dr., Department of Botany, Loikaw University

areas with an altitude between 550m and 1950m. *Tithonia* is 2-3m in height with upright and sometimes ligneous stalks in the form of woody shrubs. The large showy flowers are yellow to orange colored and -15cm wide and 10-30 cm, simply or mostly 3-7 lobed, somewhat glandular and slightly grayish beneath. The seed are spread by wind. The leaves of the plant alternate in sides they grown on which is where the plant gets the name *diversifolia*. This is accompanied by flowers which are yellow in color. *Tithonia* spp. si well known for sesquiterpene lactones and diterpenoids some of which have biological activities against insects. Most bioassays have been conducted using extracts so are not specific about which compounds are responsible for effects. In Uganda, farmers use it in field and storage pest management although there is no published work to report evidence for these effects. Medicinal - Infusion suitable for constipation, stomach pains, indigestion, sore throat, liver pains and to treat malaria. Also reported activities as anti-inflammatory, analgesic, antimalarial, antiviral, antidiabetic, antidiarrheal, antimicrobial, antispasmodic, vasorelaxant and cancer-chemopreventive (Website,2)

Tithonia diversifolia (Hemsl.) A. Grays can be used as a green fertilizer for farmers. It has the ability to restore phosphorus in high amount to the soil. It is widely cultivated as an ornamental shrub and for its medicinal value in different regions where it is commonly known as Mexican sunflower or tree marigold (Hui,Tang, 2009).

In folk medicine, the aerial parts of *Tithonia diversifolia* (Hemsl.) A. Grays are of value for the treatment of diabetes, malaria and concerning the phytochemical analysis, the nonvolatile fractions of *Tithonia diversifolia* (Hemsl.) A. Grays are a rich source of flavonoids and sesquiterpene lactones while the essential oil comprises predominantly monoterpene hydrocarbons, such as b-ocimene, a-pinene and limonene (Hui, Tang, Go, 2009).

In this paper, morphological and microscopial characters of fresh specimens, preliminary phytochemcial tests of powdered inflorescence of *Tithonia diversifolia* (Hemsl.) A. Grays, were carried out. The aim of the present was studied to identify the plant of *Tithonia diversifolia* (Hemsl.) A. Grays, to examine the microscopical characters of leaves and to study the phytochemical tests of the inflorescence.

Materials and methods

Botanical Studies

The specimens of *Tithonia diversifolia* (Hemsl.) A. Grays were collected from Loikaw Township, Kayah State. After collection, the specimens were identified with the help of available literatures Hooker (1885), Kirtikar and Basu (1935), Dutta (1979), Dassanayake (1999). Both the vegetative and reproductive parts of the fresh specimens were used for the morphological and microscopical studies.

For microscopical studies, lamina, midrib and petiole were examined by preparing free hand sections from the fresh specimens, according to the methods by Esau (1965), Metcalfe and Chalk (1950), Trease and Evans (1978), Pandey (2004) and Tandon (2011).

The samples were washed and dried at room temperature and then crushed into powder to study the powdered characteristic. Chloral hydrate solution was used as a clearing reagent. The presence of calcium oxalate crystals and prisms were tested by 80% sulphuric acid, acetic acid (BP). Solution of phloroglucinol with concentrated hydrochloric acid was tested for lignin.

Chemical Studies

The preliminary phytochemical study on the inflorescences of *Tithonia diversifolia* (Hemsl.) A. Grays had been undertaken. The experiment was carried out to determine the presence or absence of alkaloid, α - amino acid, carbohydrate, flavonoid, glycoside, phenolic compound, reducing sugar, saponin, starch, steroids, terpenoids and tannin, according to the method of British Pharmacopoeia (1968) and Marini Bettalo *et.al*, (1981).

Preliminary phytochemical test

For preliminary phytochemical investigation, the collected inflorescences 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.

Extraction

3 gm of dried powdered inflorescences of *Tithonia diversifolia* (Hemsl.) A. Grays was extracted with 100 ml of two different solvents like methanol and aqueous respectively.

Results

Botanical Studies

Morphological characters of *Tithonia diversifolia* (Hemsl.) A. Grays

Scientific name	:	*Tithonia diversifolia* (Hemsl.) A. Grays
Myanmar name	:	Taung-Nay -Kya
English name	:	Tree marigold
Family	:	Asteraceae
Flowering and fruiting period	:	November- February
Distribution	:	Wildly grows throughout Myanmar, especially at the Road sides
Part used	:	Inflorescence

Perennial robust shrubs; Leaves simple, opposite, the opposite pair unequal at each node, exstipulate, petiolate, exstipulate, petiolate; Inflorescence terminal and solitary head; Involucral bracts 2-4, free; Ray florets, unisexual, zygomorphic, sterile 12-14 per head, Disc florets, inbundibuliform, bisexual, actinomorphic, numerous, hairy; Stamen 5, epipetalous, inserted, filament filiform, free, anther syngenesious; Carpels 1, ovary oblong, inferior, unilocular, one ovule in each locule, basal placentation, style linear, long, slightly hair at the apex.

Microscopica Characters of leaves of *Tithonia diversifolia* (Hemsl.) A. Grays

Lamina

In surface view, the epidermal cells of both surfaces were parenchymatous cells, thin-walled and compactly arranged. Anticlinal walls of the lower surface were wavier than the upper ones. Stomata were slightly present on upper surface and abundant in lower surface. They were anomocytic type. Stomata were elliptic in shape with very small pores; guard cells were reniform shape with chloroplast. Multicellular trichomes were present on both surfaces.

In transverse section, the lamina was dorsiventral and smooth cuticle present on both surfaces. The upper epidermal cell was one layer on both sides, rectangular in shape and the lower epidermal cells were barrel shaped. The mesophyll was composed of palisade and spongy parenchyma. The palisade mesophyll was made up of two layer of vertically elongated cylindrical cells, which were closely packed with one another compactly arranged. The spongy mesophyll consisted of 3-4 layers of

cells, irregular to isodiametric shape and loosely arranged. Glandular and multicellular trichomes were presented on both surfaces of lamina.

The vascular bundle of lateral veins consisted of xylem always lying towards the inner side and phloem always towards the outer. This arrangement was collateral and closed type. The phloem cells were very small.

Midrib

In surface view, the epidermal cells were parenchymatous and compactly arranged and irregular. Multicellular trichomes were present. In transverse section, convex at both sides covered with thin cuticle. Both epidermal cells were rounded shaped. The lower epidermal cells were similar to those the upper epidermal cells. Below the epidermis, the cortex was differentiated into collenchyma and thin-walled parenchyma cells. The angular types of collenchymatous cells were 6-7 layers in thickness towards the upper surface and 3-4 layers in thickness towards the lower surface. They were rounded to isodiametric in shape. The parenchyma cells were 6 to 7 layers in thickness above the vascular bundle and 7 to 8 layers in thickness below the vascular bundle. They were thin- walled and irregularly rounded or oval in shape. The vascular bundle was rounded or oval in outline, collateral and closed type. The four vascular bundles were small and the bigger one was located at its lower side of midrib.

Petiole

In surface view, the epidermal cells were parenchymatous, thin-walled and mostly rectangular in shape and elongated along the length of the petiole. Glandular and non-glandular trichomes were abundant. In transverse section, the petiole was oval in outline. The cuticle layer was thick. The epidermal cells were rounded-shaped. Glandular and non-glandular trichomes were present. The cortex was differentiated into collenchyma and thin-walled parenchyma cells. The angular collenchymatous cells were 4-5 layers in thickness towards the upper surface and 3-4 layers in thickness towards the lower surface. The parenchyma cells were 16 to 17 layers in thickness above the vascular bundle and 6 to 7 layers in thickness below the vascular bundle. The parenchyma cells were oval to isodiametric in shape. The vascular bundles were embedded in the parenchymatous tissues. Vascular bundles were collateral and surrounded by a bundle sheath. The xylem was present in the upper side and the phloem was present in the lower sides.

Fig. (1) Habit

Fig. (2) Upper surface view of leaves

Fig. (3) Lower surface view of leaves

Fig. (4) Inflorescence

Fig. (5) rays Florets

Fig. (6) Disc florets

Fig. (6) L.S of Flowers

Fig. (7) T.S of ovary

Microscopical characters of leaves of *Tithonia diversifolia* (Hemsl.) A. Grays

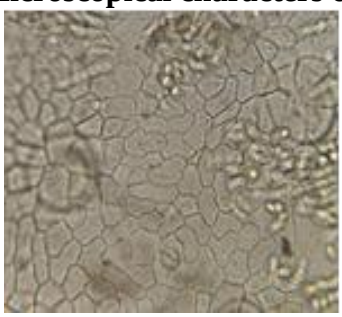

Fig. (9) Upper surface view of lamina (x 400)

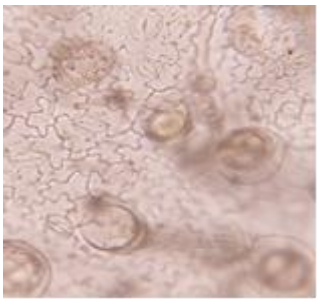

Fig.(10) Lower surface view of lamina (x 400)

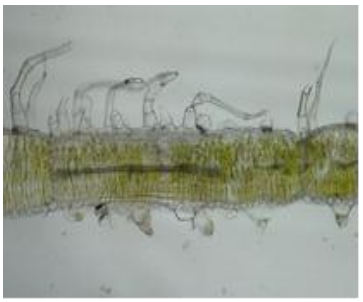

Fig.(11) T.S of lamina (x 100)

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

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

Fig. (14) surface view of petiole (x 100)

Fig.(15) T.S of petiole (x 40)

Microscopical characters of leaves of *Tithonia diversifolia* (Hemsl.) A. Grays

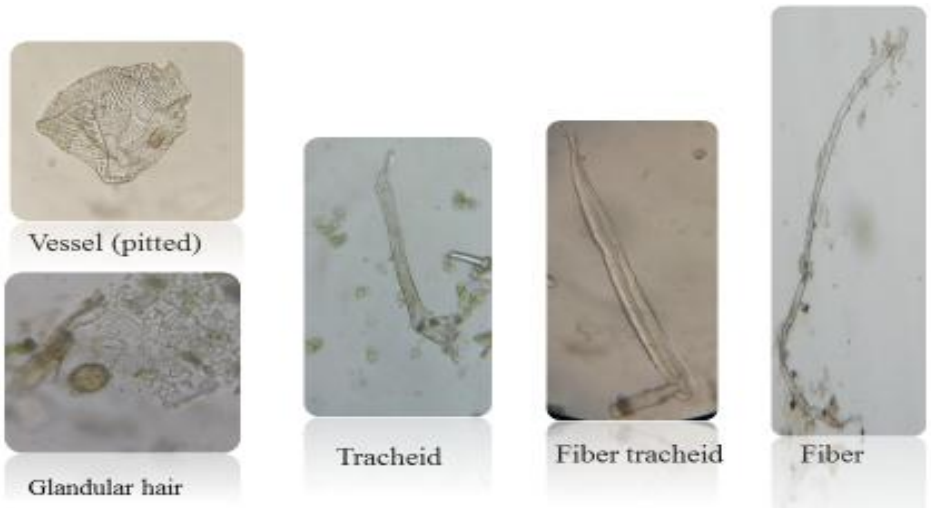

Fig. (16) Diagnostic characters of Powdered inflorescence of *Tithonia diversifolia* (Hemsl.) A. Grays

Fig. (17) Preliminary phytochemical test of leaves of *Tithonia diversifolia* (Hemsl.) A. Grays

Preliminary phytochemical test of leaves of *Tithonia diversifolia* (Hemsl.) A. Grays

The preliminary phytochemical investigation of the inflorescence of *Tithonia diversifolia* (Hemsl.) A. Grays indicated the presence of alkaloid, flavoids, phenolic compound, reducing sugar, starch, tannin, α-amino acid, saponins, terpenoids and steroids were present and carbohydrate and glycoside compound were absence.

Table (1) Preliminary phytochemical test of leaves of *Tithonia diversifolia* (Hemsl.) A. Grays

No.	Test	Extract	Test Reagent	Observation	Result
1.	Alkaloids	MeOH MeOH	1. 1% HCl + Mayer's reagent 2. Dragendorff's reagent	white ppt brown ppt	+ +
2.	Glycosides	MeOH	1ml H_2O + NaOH	Yellow green color	_
3.	Flavonoids	MeOH	Conc: HCl + Mg burning	Reddish brown color	+
4.	Phenolic compounds	MeOH	10% $FeCl_3$	Blackish color	+
5.	Steroids / Terpenoids	MeOH	$CHCL_3$ + Conc: H_2SO_4	Green color	+
6.	Carbohydrates	DW	5%α-napthol,Conc: H_2SO_4	Brick red ppt	-
7.	Reducing sugar	DW	1 ml H_2O and mixture equal part fehling's A and B	Brick red ppt	+
8.	Starch	DW	Iodine solution	Blue black ppt	+
9.	Saponins	DW	Shaken with 2ml of Distilled water	Frothing	+
10.	Tannins	DW	5%FeCl3 + H_2SO_4 (conc)	Yellowish brown ppt Reddish brown ppt	+
11.	α-amino acids	DW	Ninhydrin reagent	Pink spot	+

+ = present - = absent ppt = precipitate

Discussion and conclusion

In this paper, the morphological studies on both vegetative and reproductive parts of the plants, the microscopical studies of leaves and phytochemical study of *Tithonia diversifolia* (Hemsl.) A. Grays have been undertaken.

In morphological study, the plant of *Tithonia diversifolia* (Hemsl.) A. Grays are Perennial robust shrubs. The leaves are simple, opposite pair unequal at each node, exstipulate. The inflorescences are terminal and solitary head. Ray florets are unisexual, zygomorphic, sterile 12-14 per head. Disc florets are inbundibuliform, bisexual, actinomorphic, numerous, hairy. The stamens are 5, epipetalous. Ovary is superior, ovary oblong, 1-carpelled, 1- loculed, the ovule solitary and basal and the style linear. These characters are in agreement with those mentioned by Hooker (1885), Kirtikar and Basu (1935). Hutchinson (1967), Rendle (1967), Kurtz (1974), Dutta (1979) and Dassanayake (1999).

In the microscopical study, multicellular trichomes are present on both surfaces of the leaves. Stomata are absent on upper surface and abundant on the lower one. They are anomocytic type. In transverse section of petiole, the vascular bundles

are arranged as a curved line and collateral vascular bundle type. These characters are in agreement with those stated by Metcalfe and Chalk (1950), Pandey (2004) and Tandon (2011).

In preliminary phytochemical tests of the plants from two extracts such as Distilled water and Methanol indicated that the leaves of *Tithonia diversifolia* (Hemsl.) A. Grays contained the presence of alkaloid, flavoids, phenolic compound, reducing sugar, starch, tannin, α-amino acid, saponins, terpenoids and steroids were present. These characters are in agreement with those described by Kirtikar and Basu (1935) and Mohammed (1994).

In conclusion, the species of *Tithonia diversifolia* (Hemsl.) A. Grays are used as vegetables in the world. Hence, the members of the family Asteraceae are not only the medicinal plants but also the economic ones. Therefore, it is sincerely hoped that this present study can be beneficial for the future researchers.

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