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Research Article

PHARMACOGNOSTIC AND PHYTOCHEMICAL EVALUATION OF FLOWERS OF TUPISTRA NUTANS WALL

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ABSTRACT

To present detailed pharmacognostic profile of *Tupistra nutans* flower: an important plant in Indian system of medicine. The macroscopy, microscopy, physiochemical analysis, preliminary testing of the Flower for standardization was investigated. Morphological study of flower shows that it is leafy bracted compound inflorescence composed of many small (approx: 28-30 flowers) that resembles a single flower arising from a pale yellow color peduncle. Microscopic study of flowers shows that sepals are green and five in number. The 6 petals are reddish pink (or orange or pink or yellow). They are brighter on the inside than the outside and have lots of networked veins. The stamens each have an anther at the top of the filament shaft. There are numerous anthers connected with filaments. The anthers have a very light covering of pale yellow pollen grains on them. Each stamen produces hundreds of pollen grains. Contained inside of each pollen grain there are two sperm nuclei**.** There are two carpel whose stigma is sticky and 0.45 cm long. The ovary has small egg-like bits in it which are well developed. It can be concluded that pharmacognostic profile of *Tupistra nutans* flower is helpful in developing standards for quality, purity and simple identification.

INTRODUCTION

The genus *Tupistra* [Family Liliaceae] widely spread in eastern Himalayan region of the world with long strap shaped leaves borne on a rosette from stout rhizome. Inflorescence produced in late summer. The flowers are fleshy and last for some time in full bloom. Cool growing it likes moisture on its roots throughout the year. Inflorescence with buds and open flowers made into curry. Texture and taste of button mushrooms, slightly bitter. Plant is widely cultivated in village homes in the hilly regions and during the flowering season the inflorescences are sold in markets along with other vegetables. The plant exhibit a wide spectrum of folk and indigenous medical uses. Powdered root and flower decoction are taken to control diabetes [1].

Standardization of crude drug is an integral part of establishing its correct identity. Pharmacognostic evaluation of plant parts assists in standardization of quality, purity and sample identification. Hence the objective of present study is to evaluate various pharmacognostic parameters such as macroscopy, microscopy, physicochemical and phytochemical studies of the plant for establishing its standardization parameters.

MATERIALS AND METHODS

Plant material

The flower buds of *Tupistra nutans* plant for the proposed study were collected from Kalimpong, West Bengal in February 2012 and authenticated from Botanical Survey of India, Gangtok, where herbarium voucher specimen No. (**SHRC-5/02/2014-Tech**) has been deposited. Care was taken to select healthy plants and normal organs.

The collected flowers were cleaned and shade dried. Fresh samples were used for anatomical studies and dried parts were powdered, sieved and stored in an airtight container for further use.

Macroscopic and microscopic analysis

Key morphological features were observed for easy identification. Microscopic studies were carried out by using dissecting microscope (AJAY® OPTIK INDI: AJ-2. CM/L-9018771). Powder studies were carried out by using reagents and stains like iodine, potassium iodide, ferric chloride, Sudan III, ruthenium red and phloroglucinol with Con. HCl (1:1) [2, 3, 4]. All the reagents of analytical grade were procured from Hi-Media, Mumbai, India. Organoleptic characters like colour, texture, odor and taste were determined for flower powder.

Photo documentation

Photomicrographs of free hand sections and powder microscopy were taken using compound binocular microscope at different magnifications (Carl Zeiss Axio Imager M2 model) with inbuilt analogue camera (ProgRess C5- JENOPTIK). Computer images were captured using ProgRes® CapturePro 2.8- JENOPTIK optical system software.

Physiochemical analysis

Physico-chemical parameters of the powdered drug such as total ash, water-soluble ash, acid insoluble ash and sulphated ash were determined. Extractive values, moisture content of flowers were determined as per standard procedures [5, 6, 7].

Preparation of extracts and preliminary phytochemical analysis

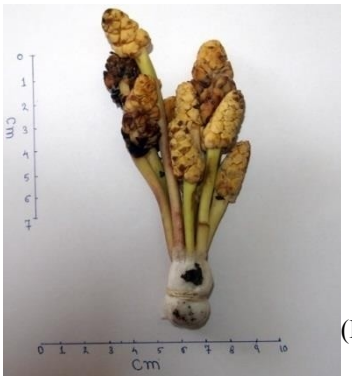
Petroleum ether (40-60°), benzene, chloroform, methanol extract obtained by successive extraction method, and water extract by maceration method. All the extracts were subjected to proximate chemical analysis [8].

RESULTS

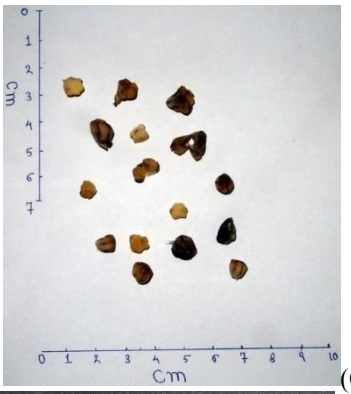
Macroscopic characteristics of flower

In those species that have more than one flower on an axis, the collective cluster of flowers is termed an inflorescence. *Tupistra nutans* inflorescence is leafy bracted compound inflorescence, composed of many small (approx: 28-30 flowers) flowers arranged in a formation that resembles a single flower. It arises in 7-9 cm pale yellow color peduncle. One bract is associated with the peduncle.

(A)



(B)



(C)

(D)

Figure 1: (A), (B), (C), (D): Inflorescence of *Tupistra nutans*

Morphological parameters

Shape: Oval

Size: Inflorescence = 9.0-**11.18**-12.1 cm length, 0.9-**1.32**-1.55 cm width.

Flower = 0.5-**0.99**-2.1 cm length, 0.4-**0.83**-1.7 cm width.

Color: yellowish pink of inflorescence

Reddish pink of flowers

Odor: None

Taste: Purely Mucilaginous (Powder also gives mucilaginous taste)

Extra Feature: Inflorescence shows 26-30 numbers of flowers.

Table 1. Data for inflorescences measurement of size (cm):

Sr. No.	Length (cm)	Width (cm)
1	12	1.5
2	10.5	1.3
3	11.4	1.2
4	9	0.9
5	12.1	1.45
6	12.05	1.55
Avg	9.0-11.18-12.1 cm	0.9-1.32-1.55 cm
	Lowest-mean-highest	Lowest-mean-highest

Table 2. Data for flowers measurement of size (cm):

Sr. No.	Length (cm)	Width (cm)
1	0.8	0.7
2	1	0.9
3	0.5	0.5
4	1	0.9
5	0.5	0.6
6	1.1	1
7	0.9	0.8
8	0.7	0.8

Microscopic characteristics of flower

Floral formula-

$K_{(5)}$ $C_{(6)}$ $A_{(6)}$ $G_{(2)}$ Capsule	**K**: Calyx: 05
	C: Corolla: 06
	A: Androceium: 06
	G: Gynaeceium: 02

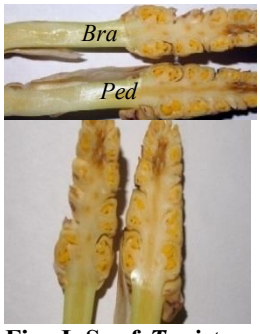


Fig: L.S. of *Tupistra nutans* Inflorescence

Fig: T.S. of *Tupistra nutans* Inflorescence

Fig: T.S. of *Tupistra nutans* Flowers

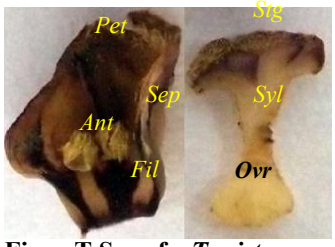
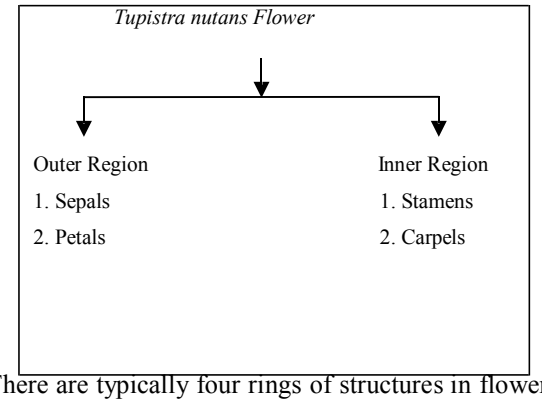


Fig: T.S. of *Tupistra nutans* Flowers

Figure 2: Transverse section of the *Tupistra nutans* Flower

(*Bra*-Bract; *Ped*-Peduncle; *Pet*- Petal; *Sep*- Sepal; Ant-Anther; *Fil*-Filament; *Stg*-Stigma; *Stl*-Style; *Ovr*-Ovary)



There are typically four rings of structures in flowers, from outside to inside they are:

1. Sepal's
2. Petal's
3. Stamen's (Anther + filament)
4. Carpel's (Ovary+Style+Stigma)

There are 5 sepals in *Tupistra nutans* flower, they are green.

The petals are reddish pink (or orange or pink or yellow). There are 6 petals. They are brighter on the inside than the outside. They are soft and smooth and have lots of networked veins. Red juice comes out of the surface when they are crushed. The flower does not really have a scent or smell or odor.

The male reproductive structures of the flower, called stamens, may be T-shaped, colored, straight or gently curved. They consist of an anther supported by filament. The stamens each have an anther at the top of the filament shaft. Pollen grains are released from the anther. Each stamen produces hundreds of pollen grains. Contained inside of each pollen grain there are two sperm nuclei**.** Stamens are very short (six numbers) compare to Carpel's in *Tupistra nutans* flower. There are numerous anthers connected with filaments. The anthers have a very light covering of pale yellow pollen grains on them.

Making up the innermost ring of structures is carpels. A carpel is a floral structure enclosing an egg in angiosperms, typically divided into ovary, style, and stigma. There is two carpel in *Tupistra nutans* flower. The stigma is sticky and 0.45 cm long. The ovary has small egg-like bits in it. They are well developed.

Figure 3. Powder characteristics of *Tupistra nutans* Flower

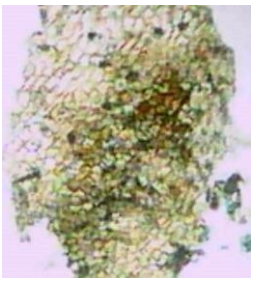


Fig: Epidermal Cells


Fig: Lignified fibres in pink color

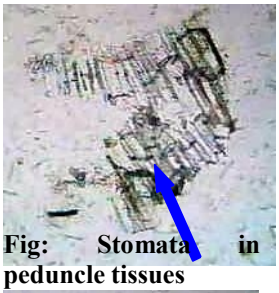


Fig: Stomata in peduncle tissues


Fig: Ground tissues of the corolla


Fig: Anther and Pollen grains


Fig: Lignified Xylem Tissue


Fig:Ca Oxalate crystal

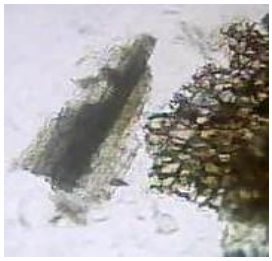


Fig: Lignified tissues in pink color


Fig:Non lignified fiber


Fig: Oil globule

Table 3. Physicochemical analysis

Sr. No.	Physical Standard		Results
			(%W/W)
1	Ash Values	Total Ash	09.33 ± 0.22
	(As per Ayurvedic Pharmacopoeia)	Acid Insoluble Ash	1.63
		Water Soluble Ash	7.46
2	Extractive values	Pet. Ether	01.05 ± 0.07
	(As per Ayurvedic Pharmacopoeia)	Water soluble	08.10 ± 0.31
		alcohol soluble	37.00 ± 1.03
3	Moisture content	Standard value	9.35 ± 0.12
	(As per Ayurvedic Pharmacopoeia)	{NMT 10% W/W}	

Phytochemical analysis

Table- 4. Successive Extractive Values of Powder *Tupistra nutans* ***flowers***

Extractives	*Tupistra nutans* ***flowers***	
	% w/w	Consistency
Pet. ether (40-60°)	0.29	Sticky
Benzene	0.73	Sticky
Chloroform	2.69	Sticky
Methanol (95 %)	17.83	Very Sticky
Water	2.91	Solid

Table-5. Preliminary Phytochemical Investigation of *Tupistra* nutans flowers

Solvent / Extract	Alkaloid	Carbohydrate	Phytosterols	Triterpenoids	Glycoside	Phenolics	Tannins	Proteins	Mucilage
Petroleum ether [40-60°]	-	-	+	-	-	-	-	-	-
Benzene	-	-	-	+	-	-	-	-	-
Chloroform	+	-	+	-	+	+	-	-	-
Methanol	-	+	-	+	+	+	+	-	+
Water	-	+	-	-	+	+	-	+	+

The Methanol extract shows presence of Glycosides, so the specific tests of Glycosides for *Tupistra nutans* ***flowers*** were performed given in **Table-6**.

Table-6. Specific Tests of Glycosides *Tupistra nutans* ***flowers***

Glycosides	*Tupistra nutans* ***flowers***
	Methanol extract
Cardiac	-
Antraqinone	-
Cynogenetic	-
Coumarin	-
Flavonoid	+
Saponins	+

'+' test is positive; '-' test is negative

DISCUSSION

The evaluation of a crude drug is an important diagnostic character useful in determining authenticity and identifying adulteration. As there is no pharmacognostic work recorded on this medicinally potent plant, the present work was undertaken to lay down the standards which could be useful for establishing its authenticity. Pharmacognostic parameters like macroscopic and microscopic features of flower have been studied. Preliminary phytochemical screening reveals the useful findings about chemical nature of drugs. Total ash values and extractive values are useful in identification and authentication of the plant material .Extractive values is useful to evaluate the chemical constituents of crude drug.

Preliminary phytochemical screening ascertains presence of phytosterols in petroleum ether and triterpenoids in benzene extract. Alkaloid, phytosterols, glycosides & phenolics in chloroform extract. Carbohydrates, triterpenoids, glycosides, phenolics, tannins in methanol extract and proteins, glycosides, carbohydrates and flavonoids in aqueous extract of the plant flower extracts. Morphological study of flower shows that it is leafy bracted compound inflorescence composed of many small (approx: 28-30 flowers) that resembles a single flower arising from a pale yellow color peduncle. Microscopic study of flowers shows that sepals are green and five in number. The 6 petals are reddish pink (or orange or pink or yellow). They are brighter on the inside than the outside and have lots of networked veins. The stamens each have an anther at the top of the filament shaft. There are numerous anthers connected with filaments. The anthers have a very light covering of pale yellow pollen grains on them..

Each stamen produces hundreds of pollen grains. Contained inside of each pollen grain there are two sperm nuclei**.** There are two carpel whose stigma is sticky and 0.45 cm long. The ovary has small egg-like bits in it which are well developed. In conclusion, the pharmacognostic standards for flowers of *Tupistra nutans* are set in present work. Set standards could be used tool for standardization of this medicinally useful plant.

CONCLUSION

The parameters studied can be utilized in identification of *Tupistra* nutans in crude drug form and can be used as a potential source for useful therapeutics. The resulted data will be beneficial for quantitative and qualitative standardization of genuine drug in herbal preparations. Positive result for alkaloids, terpenoids, glycosides and phenols is indicative of scope for future analysis.

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