Standardization on *Thevetia neriifolia*

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**Mini Review**

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**ABSTRACT**

*T. neriifolia* is a mortal plant after ingestion. The review was done to acknowledge the determination of the plant. All parts of these plants are toxic, and contain a variety of cardiac glycosides including nerifolin, thevetin A, thevetin B and oleandrin. Ingestion of oleander results in nausea, vomiting, abdominal pain, diarrhea, disrhythmias and hyperkalemia. In most cases; clinical management of poisoning by either *N. oleander* or *T. peruviana* involves administration of activated charcoal and supportive care.

Medicinal plants use is widespread. The production of medicines and the pharmacological treatment of diseases began with the use of herbs. Plants historically have served as models in drug development for some major reasons.

*T. neriifolia* is an evergreen tropical shrub or small tree. Its native range is tropical America. The plant is close relative of *Nerium oleander*, giving it one common name as yellow oleander. The plants are spread by seed. It belongs to the family Apocynaceae.

It occurs in agricultural areas, urban areas etc. it can survive most of the soils. Its leaves are willow – like, linear, lanceolate & glossy green in color[1-8] (Figure 1).

![Figure 1: Tree of T. Neriifolia](image)
The leaves contain thevetin B and digitoxigenin-β-gentiobiosyl (1→4)-α-L-acoprioside, 19-carboxydigitoxigenin-β-gentiobiosyl(1→4)-α-L-thevetoside, thevetin A, cannogenin-β-gentiobiosyl(1→4)-α-L-acofrioside, and cannogenin-α-L-rhamnoside, uzarigenin-β-gentiobiosyl(1→4)-α-L-thevetoside, and thevetogenin-β-gentiobiosyl(1→4)-α-L-thevetoside. Thevetigenin-β-gentiobiosyl(1→4)-2-O-acetyl-α-L-thevetoside, thevetogenin-β-gentiobiosyl(1→4)-α-L-acofrioside, thevetogenin-β-glucoside (1→4)-α-thevetoside are some of the glycosides which were identified in the polar fraction of the frozen leaves. The leaves also contain peruvoside so far reported from seeds only and lupeol acetate. Perivoside is a rapidly acting cardiac drug having dependable absorption from gastrointestinal tract and higher therapeutic index than digoxin; it has been introduced under the name ENCORDIN in Germany. Besides, neriifolin, neriifoside and solanoside have also been isolated from the leaves [9-15] (Table 1) (Figures 2-5).
Its stem is green turning silver/grey. Its flowers are 3 inches (7.6 cm) long, have 5 overlapping petals that open in a spiraled pin wheeled. It is long funnel shaped sometimes fragrant yellow in color. Flowers bloom from summer to fall. Its fruit is deep red – black in color. The broken foliage oozes toxic white latex sap. The plants are spread by seeds\[16,17\]. The roots of the plant bearing white colored flowers contain the triterpene alcohols, lupenol, α-β-amyrin, and taraxerol\[18-20\]. All parts of plant are highly toxic in nature but inspite of that some of the glycosides are useful in curing cardiac arrest, menstrual problems, seizures, skin problems etc. if taken in a low dose\[21-24\]. In case if a 12 kg weighted child ingest 5gm to 6gm of leaves then he may die\[25-28\].

Different standardization techniques have been done on the leaves of *Thevetia nerifolia*, they are:
1. Fluorescent analysis
2. Extractive value
3. Ash value
4. Chromatographic techniques
5. Refractive index
6. Optical rotation
The extraction was done by all such type of techniques [29-37]. After that the drug was determined. The technique is known as Maceration[38-43]. Ash value was also determined by the quality and purity of a crude drug, especially in the powdered form.

The fluorescent analysis was also determined in visible light, short UV light and longer UV light and lastly found great results in all three.

**CULTIVATION**

Yellow oleander, *Thevetia peruviana* is a bushy shrub native to tropical America. Seedlings can be found on cultivated ground and ex-pasture land. It is cultivated in gardens as an ornamental where it competes with garden plants. It is an opportunistic plant and is found growing on disturbed habitats. Its fruit, seeds, foliage or sap are poisonous to man and animals. It is a significant weed in Timor-Leste and is listed as a Category 1 weed in South Africa. In frost prone areas it is container plant, in the winter season brought inside a greenhouse or as a house plant. It tolerates most soils and is drought tolerant[44-46].

**MACROSCOPIC CHARACTERS**

A large spreading shrub usually 2.5 - 3.5m tall.

**Flowers:** The sweetly perfumed flowers are funnel-shaped, bright yellow or peach colored, 5.5 - 7cm long and 2.5 - 4cm wide, grouped in clusters at the end of the branches.

**Leaves:** The leaves are glossy green, strap-like, alternating on the stem, 5.5 - 15cm long and 0.5 - 1.5cm wide.

**Fruit/Berries:** The fruit are angular, green when immature, ripening black, broader than long and somewhat triangular in outline, with a raised ridge around the middle, about 3 - 4.5cm in diameter. The fruit is fleshy and soft like a plum and encloses a “stone” comprising two starchy kernels.

**Other:** The sap is milky.

**Symptoms:** All parts of the plant are toxic if eaten, particularly the fruit and seeds. This species has been responsible for the deaths of several children. Symptoms may include a burning sensation in the mouth, vomiting, diarrhea, dizziness and a slow or irregular heartbeat. The sap may be an irritant and cause dermatitis or blistering[47].

**MICROSCOPICAL CHARACTERS**

The histology of the xenomorphic leaves of *Allamandanerüfolia*, *Thevetia peruviana* and *Vinca minor* have been examined by LM and SEM. The most important xeromorphic characters in the leaves are thick and cutinized outer cell walls of the upper epidermis, a thick and in *A. nerüfolia* highly sculptured cuticle and in *V*, minor a two-layered palisade chlorenchyma and fiber bundles in the veins. A considerable variation occurs in characters not considered xeromorphic. The oxalate crystals in *A. nerüfolia* and *T. peruviana* differ conspicuously from each other both in shape and location. The nonarticulatedlaticifers are branched and located on both sides of the vascular strands in *A. nerüfolia* and *T. peruviana*, while they are unbranched and confined to the abaxial side of the veins in *V. minor*[48-55] (Figure 6).

![Figure 6: Microscopic Characters of *T. neriifolia*](image)
In addition attention was paid to the petiole appendages observed in the three species. When young, these formations are secretory in A. nerifolia and T. peruviana and consequently constitute colleters.

CHEMICAL CONSTITUENTS

DeVry as early as 1863 extracted from the kernels 57% of their weight of fatty oil and isolated from the mare a water-soluble glycoside to which he gave the name thevetin$^{55-60}$ (Figure 7).

![Thevetin](image)

Figure 7: Thevetin

The leaves contain thevetin B & digitoxigenin-β-gentiobiosyl (1→4)-α-L- acoprioside: 19- carboxydigitoxigenin-β-gentiobiosyl-(1→4)-α-L-thevetoside, thevetin A, cannogenin – β-gentiobiosyl-(1→4)-α-L-acofrioside, & cannogenin – α-L-rhamnoside, uzarigenin-β-gentiobiosyl-(1→4)-α-L-thevetoside & thevetogenin-β-gentiobiosyl-(1→4)-α-L-thevetoside, & thevetogenin-β-gentiobiosyl-(1→4)-2-O-acetyl-α-L-thevetoside, thevetogenin-β-gentiobiosyl-(1→4)-α-L-acofrioside, thevetogenin-β-glucoside (1→4)-α-thevetoside are some of the glycosides which were identified in the polar fraction of the frozen leaves$^{61-65}$.

The leaves also contain peruvoside so far reported from seeds only and lupeol acetate. Perivoside is a rapidly acting cardiac$^{66,67}$.

SPECIAL CHARACTERISTICS

Poisonous – All parts of the plant and especially the seeds are highly poisonous and contain toxic cardiac glycosides like Thevetin and Peruvoside that can cause severe illness or even death if ingested. The sap may cause skin irritation. Burning the wood or plant material can produce toxic smoke$^{68,73}$.

FLOURESCENT ANALYSIS DETERMINATION

Fluorescence light shows different colors in different lights by adding different chemicals in the extracted compound$^{73-77}$ (Table 2) (Figures 8-10).

1. Fluorescence analysis in visible light
2. Fluorescence analysis in short UV light
3. Fluorescence analysis in long UV light

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ASH VALUE DETERMINATION

Ash values are helpful in determining the quality & purity of a crude drug, especially in the powdered form. On incineration, crude drugs normally leave an ash usually consisting of carbonates, phosphates and silicates of sodium, potassium, calcium & magnesium. The total ash of a crude drug reflects the care taken in its preparation. A higher limit of acid - insoluble ash is imposed, especially in cases where silica may be present or when the calcium oxalate content of the drug is very high. Mixing if sulphuric acid with the powdered crude drug before ashing & this sulphated ash is normally less fusible than ordinary ash [78-83].

The total ash value when determined it was found to be 7% w/w.
In case of acid insoluble ash value it was found to be 1.6% w/w. The loss of drying (12% w/w) was also determined in the acid insoluble ash determination [84].

**EXTRACTIVE VALUE DETERMINATION**

Extractive value of crude drugs are useful for their evaluation, especially when the constituents of a drug cannot be readily estimated by any other means. Further, these values indicate the nature of the constituents present in a crude drug. Extractive value were found to be 21.6% for alcohol. Extractive value were found to be 20% for water [85-89].

**CHROMATOGRAPHIC TECHNIQUES**

Chromatography is a separation of process that is achieved by distributing the substances to be separated between two phases, a stationary phase and a moving phase. Those substances distributed preferentially in the moving phase will pass through the chromatographic system faster than those that are distributed preferentially in the stationary phase [90-95]. There are different types of chromatographic techniques which are as follows:

1. Paper chromatography
2. High performance liquid chromatography
3. Gas chromatography
4. High performance thin layer chromatography
5. Gas liquid chromatography
6. Ion exchange chromatography
7. Liquid chromatography
8. Affinity chromatography
9. Gel chromatography
10. Moving bed chromatography
11. Column chromatography [95-98]

\[ \text{Rf} = \frac{\text{Distance travelled by Component}}{\text{Distance travelled by Solvent}} \]

Rf value for a compound is a constant or a particular solvent system. This value is used for the identification of the compound and is compared with the reference standard.

T. neriifolia, is the drug in which TLC had been performed on it.

The TLC plate was kept in Hot Air Oven for 1 hour at 90-100°C to activate the plate. Then 18g of Silica gel was weighed and dissolved using distilled water. The silica gel past was then poured evenly on the TLC plate to form a thin layer. The TLC plate was kept in hot air oven for 1 hour at 90-100°C to dry. The evaporated sample was loaded in the gel and allowed to run in the solvent-ethanol. The sample, which was runned in the TLC was removed. Ethanol was added to the powdered form of sample that contains silica gel. Then it was centrifuged at 5,000rpm for 10 minutes. The supernatant was collected to obtain the purified sample [99-101].

**Refractive Index**

It is a ratio of velocity of light in vacuum to velocity in the substance. It is a physical constant & very useful for standardization. it is measured by Refractometer [102-106].

\[ \text{RI} = \frac{\text{Volume of light in vaccum}}{\text{Volume of light in substance}} \]

**OPTICAL ROTATION**

It is determined with the help of Polarimeter [107-109]. If the rotation of plane of polarized light is towards right, it is known as Dextrorotatory (+) & if it is towards left it is known as Levorotatory (-) [110].
MICROBIAL CONTAMINATION

*Thevetia neriifolia* is mainly used as a pest control. As it is also used as an anti-termite\(^{111-114}\).

SPECTROSCOPIC ANALYSIS

*T. neriifolia* have the capacity to absorb vibration by specific wavelength. It is very useful parameter for evaluation of *T. neriifolia*. Various phytochemicals are determined by spectroscopic drug analysis (UV, IVR, NMR and Mass)\(^{115-118}\).

**UV**: Specific absorbent are used in this.
**IVR**: Functional groups of biomolecule.
**NMR**: Position of protons.
**Mass**: molecular weight of compound.

ANALYSIS OF PHYTOCHEMICAL COMPONENTS

7g of fresh *Thevetia peruviana* leaves were collected, weighed and allowed to dry. Then it was powdered using motor and pestle. The powdered leaves were homogenized with 20ml of ethanol. Along with 20ml of ethanol, 50ml of ethanol was added and 50ml of Distilled water was added and poured into the separating funnel. The separating funnel was kept overnight to separate the extract into aqueous phase and solid phase. Then the aqueous phase was collected, filtered and centrifuged. The centrifuged sample was allowed to evaporate for further use\(^{119}\).

PROTEIN ANALYSIS

The extracts were analyzed for the presence of protein by using TLC. The evaporated sample was loaded in the thin layer formation of silica gel in the TLC plate. Then, it was placed in the beaker which containing ethanol and allowed to run. After running, the ninhydrin was sprayed over the sample and again the plate was kept in hot air oven for 20 minutes to observe the color change. Pink or purple color formation indicates the presence of protein.

IODINE TEST FOR PHENOL

Thin layer was formed with the help of silica gel on TLC plate. The sample was loaded in the gel and allowed to run in acetic acid and chloroform (1:9). After 10 minutes, it was removed and place in a glass beaker containing iodine crystals. The color change indicates the presence of phenols and alkaloids.

BORNTRAGER'S TEST

To the extract, the chloroform was added and mixed well. To the separated layer of chloroform, the ammonia solution was added. The color change indicates the presence of glycosides.

GC-MS (GAS CHROMATOGRAPHY-MASS SPECTROMETRY)

GC/MS is a technique that can be used to separate volatile organic compounds (VOCs). We can combine GC or MS with other separation and analytical techniques. The Gas Chromatography/ Mass Spectrometry (GC/MS) instrument separates chemical mixtures (the GC component) and identifies the components at a molecular level (the MS component). It is one of the most accurate tools for analyzing environmental samples. The GC works on the principle that a mixture will separate into individual substances when heated. The heated gases are carried through a column with an inert gas (such as helium). As the separated substances emerge from the column opening, they flow into the MS. Mass spectrometry identifies compounds by the mass of the analyte molecule. A "library" of known mass spectra, covering several thousand compounds, is stored on a computer. Mass spectrometry is considered the only definitive analytical detector. The purified sample was used for GC/MS to analyze the compounds present in the sample.

CONDITIONS

- **Column Oven Temperature**: 70˚c
- **Injector Temperature**: 200˚c
- **Injection Mode**: split
- **Split Ratio**: 40
- **Flow Control Mode**: Linear Velocity
- **Column Flow**: 1.51ml/min
- **Carrier Gas**: Helium 99.9995%
- **Purity**
ESTIMATION OF GLYCOSIDES

The estimation of glycosides was performed by using Baljet’s reagent into the sample and the odd value was taken at 495 nm using spectrophotometer to know the amount of glycosides present in the sample.

TOXICITY OF T.NERIIIFOLIA

T.neriifolia plants are found to be toxic in its nature. The toxins are cardenolides called Thevetin A and Thevetin B. others include Peruvoside, Neriifolin, Thevetoxin and Ruvoside. These cardenolides are not destroyed by drying or heating and they are very similar to digoxin from Digitalis purpurea. They produce gastric and cardio toxic effects. Antidotes for treatment include atropine and Digoxin antibodies and treatment may include oral administration of activate charcoal. Ovine polyclonal anti-digitoxin Fab fragment antibody can be used to treat digoxin poisoning. During the toxic studies it was found that Thevetin A and B cause poisonous in the plant in high amount and due to these constituents only death may occur even at a low dose too. Still researches are going on to reduce the toxicity of the plant.

Thetetia peruviana plants are toxic to most vertebrates as they contain cardiac glycosides. Many cases of intentional and accidental poisoning of humans are known. A few bird species are however known to feed on them without any ill effects. These include the Asian Koel, Red-whiskered Bulbul, White-browed Bulbul, Red-vented Bulbul, Brahminy Myna, Common Myna and Common Grey Hornbill. The toxins are cardenolides called Thevetin A and Thevetin B (Cerebroside), others include peruvoside, neriifolin, thevetoxin and ruvoside. These cardenolides are not destroyed by drying or heating and they are very similar to digoxin from Digitalis purpurea. They produce gastric and cardiotoxic effects. Antidotes for treatment include atropine and Digoxin antibodies and treatment may include oral administration of activate charcoal., Ovine polyclonal anti-digitoxin Fab fragment antibody (DigiTAb; Therapeutic Antibodies Inc.) can be used to treat digoxin poisoning, but for many countries the cost is prohibitive.

Thetetia cardiac glycosides can lead to intoxication, thus they are important indicators for forensic and pharmacologic surveys. Among cardiac glycosides, this paper also found thevetin C from the seeds of T.neriifolia which is functionally different from thevetin A and thevetin B. The absence of an unsaturated lactone ring renders the glycosides cardio-inactive [120].

CONCLUSION

This review concluded that T. neriifolia contains number of phytoconstituents which reveals its uses for various therapeutic purposes and high amount of glycosides. In T. neriifolia cardiac glycosides is high in amount as compare to all and isfound to be most important indicator in different forensic and pharmacological activities. The Plant parts can be used for the treatment of various disorders in human being such as, diabetes, liver toxicity, fungal infection, microbial infection, inflammation, pyrexia and to relieve pain. Still a lot of research is required in the evaluation of different pharmacological activities of T.neriifolia plant.

CONFLICT OF INTEREST

Authors have no conflict of interest. Study was done on the basis of research performed in a lab scale on T.neriifolia.

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