

# Research & Reviews: Journal of Botanical Sciences

## Standardization on *Thevetia neriifolia*

Priya Kumar\*<sup>1</sup>, Deepika Chandra<sup>1</sup>

<sup>1</sup>Department of Pharmaceutical Technology, Uttar Pradesh Technical University, Noida Region, Uttar Pradesh, India

### Mini Review

Received: 05/05/2015  
Revised: 20/05/2015  
Accepted: 03/06/2015

#### \*For Correspondence

Priya Kumar, Department of Pharmaceutical Technology, Uttar Pradesh Technical University, Noida Region, Uttar Pradesh, India, E-mail: priyavarunkumar@gmail.com

Keywords: Maceration, Toxic, Spectroscopic, Chromatography.

#### 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, diarrhoea, disrhythmias and hyperkailemia.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<sup>[1-8]</sup> (Figure 1).



Figure 1: Tree of *T. Neriifolia*

The leaves contain thevetin B & digitoxigenin- $\beta$ -gentiobiosyl (1 $\rightarrow$ 4)- $\alpha$ -L- acoprioside: 19- carboxydigitoxigenin- $\beta$ -gentiobiosyl-(1 $\rightarrow$ 4)- $\alpha$ -L thevetoside, thevetin A , cannogenin -  $\beta$ -gentiobiosyl-(1 $\rightarrow$ 4)- $\alpha$ -L- acofriptide,&cannogenin -  $\alpha$ - L- rhamnoside,uzarigenin- $\beta$ -gentiobiosyl-(1 $\rightarrow$ 4)- $\alpha$ -L-thevetoside&thevetogenin- $\beta$ -gentiobiosyl-(1 $\rightarrow$ 4)- $\alpha$ -L-thevetoside , &thevetogenin- $\beta$ -gentiobiosyl-(1 $\rightarrow$ 4)-2-O-acetyl- $\alpha$ -L-thevetoside, thevetogenin- $\beta$ -gentiobiosyl-(1 $\rightarrow$ 4) -  $\alpha$ -L acofriptide,thevetogenin- $\beta$ - glucoside (1 $\rightarrow$ 4)- $\alpha$ -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. Peruvoside 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<sup>[9-15]</sup> (Table 1) (Figures 2-5).

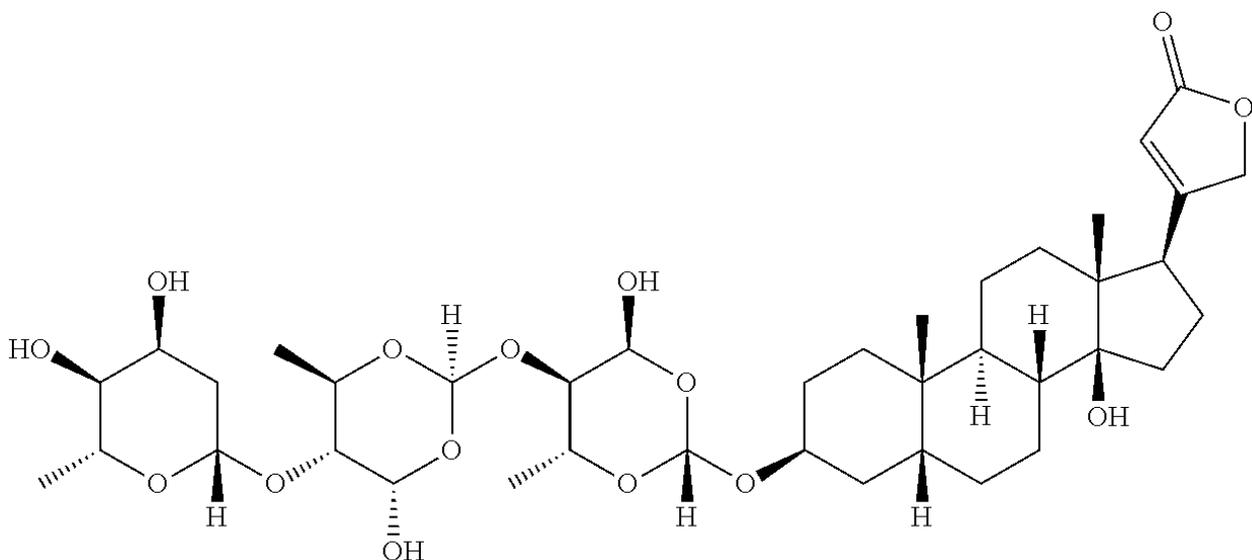


Figure 2: Thevetin A

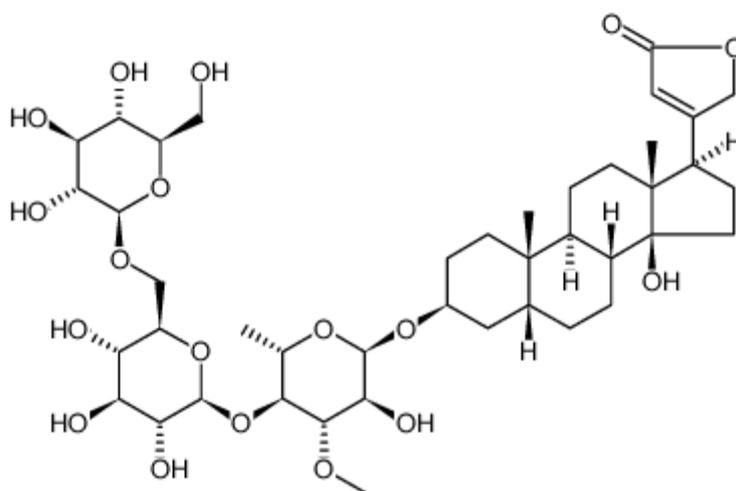


Figure 3: Thevetin B

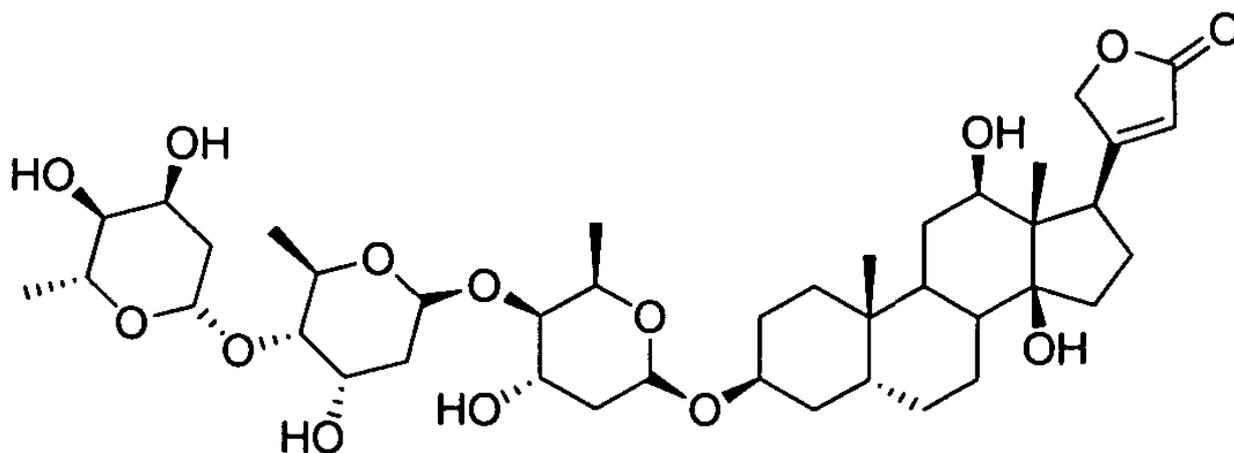


Figure 4: Peruvoside

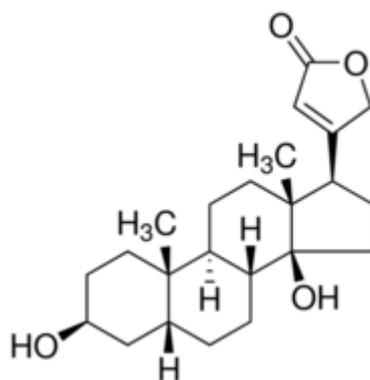


Figure 5: Digitoxigenin

### DEFINING PARTS

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<sup>[16,17]</sup>.

The roots of the plant bearing white colored flowers contain the triterpene alcohols, lupenol,  $\alpha$ - $\beta$ -amyrin, and taraxero<sup>[18-20]</sup>.

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<sup>[21-24]</sup>.

In case if a 12 kg weighted child ingest 5gm to 6gm of leaves then he may die<sup>[25-28]</sup>.

Different standardization techniques have been done on the leaves of *Thevetia neriifolia*, they are:

1. Fluorescent analysis
2. Extractive value
3. Ash value
4. Chromatographic techniques
5. Refractive index
6. Optical rotation

7. Microbial contamination
8. Spectroscopic analysis

The extraction was done by all such type of techniques [29-37]. After that the drug was determined. The technique is known as Maceration<sup>[38-43]</sup>. 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<sup>[44-46]</sup>.

### 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<sup>[47]</sup>.

### 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 nonarticulated laticifers 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*<sup>[48-55]</sup> (Figure 6).

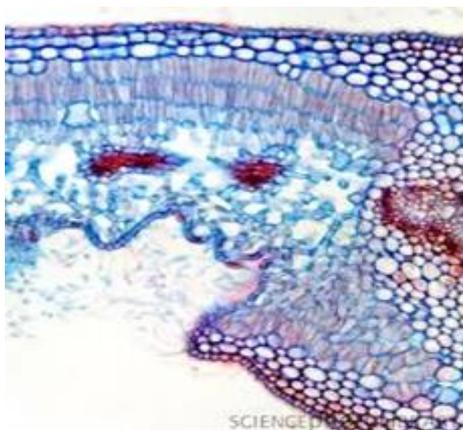


Figure 6: Microscopic Characters of *T. neriiifolia*

In addition attention was paid to the petiole appendages observed in the three species. When young, these formations are secretory in *A. nerüfolia* and *T. peruviana* and consequently constitute colleter.

### 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<sup>[55-60]</sup> (Figure 7).

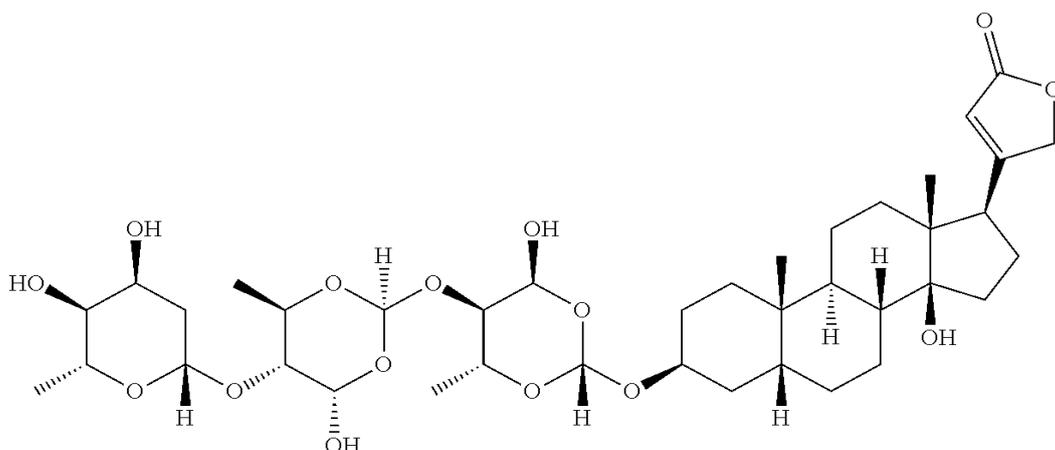


Figure 7: Thevetin

The leaves contain thevetin B & digitoxigenin- $\beta$ -gentiobiosyl (1 $\rightarrow$ 4)- $\alpha$ -L- acoprioside: 19- carboxydigitoxigenin- $\beta$ -gentiobiosyl-(1 $\rightarrow$ 4)- $\alpha$ -L thevetoside, thevetin A , cannogenin -  $\beta$ -gentiobiosyl-(1 $\rightarrow$ 4)- $\alpha$ -L- acofrioside, & cannogenin -  $\alpha$ - L- rhamnoside, uzarigenin- $\beta$ -gentiobiosyl-(1 $\rightarrow$ 4)- $\alpha$ -L-thevetoside & thevetogenin- $\beta$ -gentiobiosyl-(1 $\rightarrow$ 4)- $\alpha$ -L-thevetoside , & thevetogenin- $\beta$ -gentiobiosyl-(1 $\rightarrow$ 4)-2-O-acetyl- $\alpha$ -L-thevetoside, thevetogenin- $\beta$ -gentiobiosyl-(1 $\rightarrow$ 4) -  $\alpha$ -L acofrioside, thevetogenin- $\beta$ - glucoside (1 $\rightarrow$ 4)- $\alpha$ -thevetoside are some of the glycosides which were identified in the polar fraction of the frozen leaves<sup>[61-65]</sup>.

The leaves also contain peruvoside so far reported from seeds only and lupeol acetate. Perivoside is a rapidly acting cardiac<sup>[66,67]</sup>.

### 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<sup>[68-73]</sup>.

### FLOURESCENT ANALYSIS DETERMINATION

Flourescence light shows different colors in different lights by adding different chemicals in the extracted compound <sup>[73-77]</sup>(Table 2) (Figures 8-10).

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



Figure 8: Fluorescence analysis in visible light



Figure 9: Fluorescence analysis in short UV light



Figure 10: Fluorescence analysis in long UV light

#### **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 <sup>[78-83]</sup>.

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<sup>[84]</sup>.

### 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 <sup>[85-89]</sup>.

### 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<sup>[90-95]</sup>.

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<sup>[95-98]</sup>

$$R_f = \frac{\text{Distance travelled by Component}}{\text{Distance travelled by Solvent}}$$

R<sub>f</sub> 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<sup>[99-101]</sup>.

### Refractive Index

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

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

### OPTICAL ROTATION

It is determined with the help of Polarimeter<sup>[107-109]</sup>.

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 (-)<sup>[110]</sup>.

## MICROBIAL CONTAMINATION

*Thevetia neriifolia* is mainly used as a pest control. As it is also used as an anti- termite<sup>[111-114]</sup>.

## 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)<sup>[115-118]</sup>.

**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 <sup>[119]</sup>.

### 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. NERIIFOLIA

*T. neriifolia* plants are found to be toxic in its nature. The toxins are cardenolides called Thevetin A and Thevetin B. Others include Peruvidoside, Neriifolin, Thevetoxin and Ruvidoside. 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-digoxin 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.

*Thevetia 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 ruvidoside. 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-digoxin Fab fragment antibody (DigiTAB; Therapeutic Antibodies Inc.) can be used to treat digoxin poisoning, but for many countries the cost is prohibitive.

*Thevetia* 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<sup>[120]</sup>.

## 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 is found 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*.

## REFERENCES

1. Odhiambo PO et al. Phyto-Chemical Screening of Wild Types and Tissue Cultured Yellow Oleander *Thevetia peruviana* Pers.K.Schum in Kenya. *AdvPharmacoepidem Drug Safety*. 2012;1:120.
2. Soliás IM et al. Medicinal Potential from Plant Biodiversity in a Mediterranean Scrubland, *ThymoPiperellae-Cistetum Crispi* Costa, Peris&Stubing 1986. *J BiodiversBiopros Dev*. 2014;1:111.
3. Khajja BS et al. Forensic Study of Indian Toxicological Plants as Botanical Weapon (BW): A Review. *J Environment Analytic Toxicol*. 2011;1:112.
4. <http://esciencecentral.org/journals/2329-6836/2329-6836.S1.005-051.pdf>
5. <http://omicsgroup.org/journals/2167-0501/2167-0501-S1.002-076.pdf>
6. [http://omicsonline.org/2157-7463/2157-7463\\_S1.007\\_025.pdf](http://omicsonline.org/2157-7463/2157-7463_S1.007_025.pdf)
7. <http://omicsonline.org/2157-7625/2157-7625-S1.009-049.pdf>

8. Kayarohanam S and Kavimani S. Current Trends of Plants Having Antidiabetic Activity: A Review. *J Bioanal Biomed.* 2015;7: 055-65.
9. <http://omicsgroup.org/journals/medicinal-aromatic-plants.php>
10. *Journal of Plant Physiology & Pathology*
11. Bazie S et al. Antifungal Activity of Some Plant Extracts against (*Colletotrichum Musae*) the Cause of Postharvest Banana Anthracnose. *J Plant Pathol Microb.* 2014;5:226.
12. Kayarohanam S and Kavimani S. Current Trends of Plants Having Antidiabetic Activity: A Review. *J Bioanal Biomed.* 2015;7: 055-65.
13. <http://omicsonline.org/2155-952X/2155-952X.S1.027-006.pdf>
14. <http://omicsonline.org/2155-952X/2155-952X-S1.020-022.pdf>
15. <http://omicsonline.org/2155-952X/2155-952X-S1.020-022.pdf>
16. Mubarak NM and Faridah Y. Protein Purification in Chromatographic Media using Multiwall Carbon Nanotubes. *J Bioprocess Biotech.* 2015;5:214.
17. Ogwuche CE et al. Isolation and Biological Activity of the Triterpene B-Amyrin from the Aerial Plant Parts of *Maesobotrya Barteri* (Baill). *Med chem.* 2014;4:729-733.
18. Kazemi M and Soleiman-Beigi M. Tetra-N-Butyl Ammonium Hydroxide as Highly Efficient for the Acylation of Alcohols, Phenols and Thiols. *Organic Chem Curr Res.* 2013;2:119.
19. Caimmi S et al. False Latex Allergy and Allergy Work-up in a Child Undergoing General Anesthesia. *Pediat Therapeut.* 2013;3:180.
20. Ogwuche CE et al. Isolation and Biological Activity of the Triterpene B-Amyrin from the Aerial Plant Parts of *Maesobotrya Barteri* (Baill). *Med chem.* 2014;4:729-733.
21. Elgemeie GH et al. Design, Synthesis and Cytotoxic Evaluation of Novel Heterocyclic Thioglycosides. *Med chem.* 2014;4:814-820.
22. Elgemeie GH et al. Design, Synthesis and In vitro Anti-tumor Evaluation of Novel Acrylohydrazide Thioglycosides. *Med chem.* 2014;4:400-406.
23. Uchiumi F et al. Effect of Lignin Glycosides Extracted from Pine Cones on the Human SIRT1 Promoter. *Pharm Anal Acta.* 2013;4: 266.
24. Harry-O'kuru RE et al. Medicinal Components Recoverable From Sicklepod (*Senna obtusifolia*) Seed: Analysis of Components by HPLC-MS. *J Chromatogr Separat Techniq.* 2012;S2:001.
25. Gebreselassie N, Animut G, Urge M (2015) Effect of Supplementation with Dried Leaves of *Acacia albida*, *Acacia seyal* and their Mixture on Feed Intake and Digestibility of Local Sheep Fed Barley Straw as a Basal Diet. *J Fisheries Livest Prod* 3:124.
26. <http://omicsonline.org/2153-2435/2153-2435.S1.017-021.pdf>
27. <http://omicsonline.org/1948-5956/1948-5956-S1.026-027.pdf>
28. <http://omicsonline.org/1948-5956/1948-5956-S1.026-019.pdf>
29. Shuijun Li. Standardization of LC-MS/MS in Clinical Laboratory. *J Chromatogr Sep Tech.* 2015;6:e128.
30. <http://omicsonline.org/0975-0851/0975-0851-S1.10-003.pdf>
31. <http://omicsonline.org/2153-2435/2153-2435.S1.018-023.pdf>
32. <http://omicsonline.org/2153-2435/2153-2435.S1.016-008.pdf>
33. Maher HM. Stacking As Sample On-Line Pre-concentration Technique in Microemulsion Electrokinetic Chromatography. *J Chromatogr Sep Tech.* 2015;6:e130.
34. Chauhan A et al. Phyto-Chemical Screening and Anti Listerial Activity of *Annona muricata* (L) Leaf Extract. *J Chromatogr Sep Tech.* 2015; 6:269.
35. Wujian J et al. A Simple Protein Precipitation-based Simultaneous Quantification of Lovastatin and Its Active Metabolite Lovastatin Acid in Human Plasma by Ultra-Performance Liquid Chromatography-Tandem Mass Spectrometry using Polarity Switching. *J Chromatogr Sep Tech.* 2015;6:268.
36. Bava M et al. Qualitative-Quantitative Analysis by LC/DAD and GPC of the Polyphenols of *Vitis rotundifolia* Grape Seeds for the Development of an Industrial Nutraceutical Product. *J Chromatogr Sep Tech.* 2015;6: 266.
37. Mottaleb MA et al. Use of LCMS and GC-MS Methods to Measure Emerging Contaminants in Pharmaceutical and Personal Care Products (PPCPs) in Fish. *J Chromatogr Sep Tech.* 2015;6:267.

38. <http://omicsonline.org/2155-6156/2155-6156.S1.028-010.pdf>
39. <http://omicsonline.org/1948-5964/1948-5964.S1.022-031.pdf>
40. <http://omicsonline.org/2155-9597/2155-9597.S1.008-038.pdf>
41. <http://omicsonline.org/2155-9597/2155-9597.S1.008-035.pdf>
42. <http://omicsonline.org/2155-9597/2155-9597.S1.008-033.pdf>
43. <http://omicsonline.org/1948-593X/1948-593X.S1.015-009.pdf>
44. Holsbeeck MV et al. Overexpression of L-Arabinose Isomerase for Production of the Low-Calorie Bulk Sweetener D-Tagatose. *Enz Eng.* 2015;4:125.
45. Gerbec B et al. Solid State Cultivation of *Hericiumerinaceus* Biomass and Erinacine: A Production. *J Bioprocess Biotech.* 2015;5: 210.
46. Chernogor LI et al. Formation of Spicules During the Long-term Cultivation of Primmorphs from the Freshwater Baikal Sponge *Lubomirskiabaikalensis*. *Organic Chem Current Res.* 2011;S2:001.
47. Sani A et al. Pharmacognostic Studies of the Stem Bark of *Detarium Microcarpum* "Guill. and Perr. (Fabaceae) . *Nat Prod Chem Res.* 2014;S1:004.
48. Hao M et al. Efficacy of Transcatheter Arterial Infusion Alone or Combined with Transcatheter Arterial Chemoembolization on Advanced Hepatocellular Carcinoma. *J Cancer Sci Ther.* 2011;3: 130-133.
49. Singh G et al. Prevalence of Malaria in a Tertiary Care Hospital in Navi Mumbai, India. *J Bacteriol Parasitol.* 2015;6:221.
50. Yoshioka H et al. Usefulness of Evaluation of Nuclear Color by Visible-Microscopic Spectroscopy for Objective Differentiation between Non-Cancer and Cancer Cells Prepared Using Liquid-Based Cytology. *J Cytol Histol.* 2015;6:308.
51. <http://omicsonline.org/2157-7013/2157-7013.S1.033-024.pdf>
52. Bacchetti T et al. Carotenoids, Phenolic Compounds and Antioxidant Capacity of Five Local Italian Corn (*Zea Mays* L.) Kernels. *J Nutr Food Sci.* 2013;3:237.
53. Ghadge PN and Prasad K Some Physical Properties of Rice Kernels: Variety PR-106. *J Food Process Technol.* 2012;3:175.
54. <http://esciencecentral.org/journals/2329-6836/2329-6836.S1.005-028.pdf>
55. <http://omicsonline.org/1948-593X/1948-593X.S1.015-018.pdf>
56. <http://omicsonline.org/2157-7110/2157-7110.S1.002-014.pdf>
57. <http://omicsonline.org/2155-952X/2155-952X.S1.028-066.pdf>
58. [http://omicsonline.org/2153-0769/2153-0769\\_S1.026\\_007.pdf](http://omicsonline.org/2153-0769/2153-0769_S1.026_007.pdf)
59. <http://omicsgroup.org/journals/2168-9881/2168-9881-S1.008-007.pdf>
60. <http://omicsgroup.org/journals/2168-9881/2168-9881-S1.008-004.pdf>
61. <http://omicsonline.org/2155-952X/2155-952X-S1.020-013.pdf>
62. <http://omicsonline.org/2155-952X/2155-952X-S1.020-006.pdf>
63. <http://omicsonline.org/2155-952X/2155-952X-S1.020-026.pdf>
64. [http://omicsonline.org/2157-7110/2157-7110\\_S1.010\\_013.pdf](http://omicsonline.org/2157-7110/2157-7110_S1.010_013.pdf)
65. [http://omicsonline.org/2157-7110/2157-7110\\_S1.010\\_070.pdf](http://omicsonline.org/2157-7110/2157-7110_S1.010_070.pdf)
66. <http://omicsgroup.org/journals/2168-9881/2168-9881-S1.004-025.pdf>
67. Steele J et al. Heparin Induced Thrombocytopenia and Cardiac Surgery: A Comprehensive Review. *J Blood Disord Transfus.* 2011;S2:003.
68. Kyoon Hwang J et al. Spinnaker Sail Sign Accompanied with Pneumopericardium and Pneumoperitoneum. *J Neonatal Biol.* 2015;4:177.
69. Divekar A et al. Non-Restrictive Ductal Patency in Management of Cardiac Failure in Congenital Diaphragmatic Hernia – Non-Invasive Biventricular Assist. *J Neonatal Biol.* 2015;4:171.
70. Hasan R et al. Apical Thrombus Mimicking Cardiac Myxoma: Application of Cardiovascular Magnetic Resonance. *OMICS J Radiol.* 2015;4:181.
71. Seki Y et al. Landiolol Hydrochloride Normalizes Diminished Levels of Cardiac Vascular Endothelial Growth Factor (VEGF) Signaling System Components in Lipopolysaccharide-Induced Sepsis Independent of Inflammatory Markers. *J Vasc Med Surg.* 2015;3:193.
72. Behranwala A et al. Non-Hodgkins Lymphoma Involving Multiple Cardiac Chambers with Skeletal Muscle Involvement in a Hepatitis C Positive Patient. *J Vasc Med Surg.* 2015;3:191.
73. <http://omicsonline.org/2155-9880/2155-9880.S1.024-058.pdf>

74. <http://omicsonline.org/2155-9880/2155-9880.S1.024-059.pdf>
75. <http://omicsonline.org/2155-9880/2155-9880.S1.022-006.pdf>
76. <http://omicsonline.org/2155-9880/2155-9880.S1.024-057.pdf>
77. <http://omicsonline.org/2155-9880/2155-9880.S1.024-056.pdf>
78. <http://omicsonline.org/2155-9880/2155-9880.S1.024-055.pdf>
79. [http://omicsonline.org/2157-7110/2157-7110\\_S1.009\\_029.pdf](http://omicsonline.org/2157-7110/2157-7110_S1.009_029.pdf)
80. <http://omicsgroup.org/journals/2167-0501/2167-0501-S1.002-029.pdf>
81. <http://omicsgroup.org/journals/2167-0501/2167-0501-S1.002-028.pdf>
82. <http://omicsgroup.org/journals/2167-0501/2167-0501-S1.002-024.pdf>
83. [http://omicsonline.org/2157-7110/2157-7110\\_S1.009\\_029.pdf](http://omicsonline.org/2157-7110/2157-7110_S1.009_029.pdf)
84. <http://omicsonline.com/open-access/2247-2452/2247-2452.S1.006-061.pdf>
85. Moharamzadeh K et al. Evaluation of the Effects of Ethanol on Monolayer and 3D Models of Human Oral Mucosa. *J Environ Anal Toxicol.* 2015;5:275.
86. Kamal T. An Investigation on the Preparation of Containing Low Caloric Biscuits with Supplementation of Dietary Fiber. *Food Processing & Technology.* 2015; 6: 1-6.
87. Saharan BS and Verma S. Evaluation of Rhizospheric Bacteria from *Ocimum* sp. as Potential Pgpr. *J Microb Biochem Technol.* 2015;7:088-095.
88. Mubarak NM and Faridah Y. Protein Purification in Chromatographic Media using Multiwall Carbon Nanotubes. *J Bioprocess Biotech.* 2015;5:214.
89. Talaat W. Micellar Liquid Chromatographic Determination of Lamivudine, Indinavir and Ketoconazole in Dosage Forms and Biological Fluids. *Pharm Anal Acta.* 2015;6:327.
90. Belal F et al. Micellar Liquid Chromatographic Determination of Idrocilamide in Dosage Form and Biological Fluids: Application to Stability Study. *Pharm Anal Acta.* 2015;6:335.
91. Dasgupta TK et al. Spectroscopic & Chromatographic Methods for Quantitative Analysis of Phospholipid Complexes of Flavonoids: A Comparative Study. *Pharm Anal Acta.* 2014;6:322.
92. Chutipongvivate S et al. Alternative Malaria Diagnostic Tools: Evaluation of *Plasmodium falciparum* Detection along Thailand's Border by Loop-Mediated Isothermal Amplification (LAMP) and Immunochromatographic Test (ICT). *J Trop Dis.* 2014;2:147.
93. Maher HM. Stacking As Sample On-Line Pre-concentration Technique in Microemulsion Electrokinetic Chromatography. *J Chromatogr Sep Tech.* 2015;6:e130.
94. Chauhan A et al. Phyto-Chemical Screening and Anti Listerial Activity of *Annona muricata* (L) Leaf Extract. *J Chromatogr Sep Tech.* 2015;6:269.
95. Wujian Jet al. A Simple Protein Precipitation-based Simultaneous Quantification of Lovastatin and Its Active Metabolite Lovastatin Acid in Human Plasma by Ultra-Performance Liquid Chromatography-Tandem Mass Spectrometry using Polarity Switching. *J Chromatogr Sep Tech.* 2015;6:268.
96. Bava M et al. Quali-Quantitative Analysis by Lc/Dad and Gpc of the Polyphenols of *Uva Ursi* and *Vitis rotundifolia* Grape Seeds for the Development of an Industrial Nutraceutical Product. *J Chromatogr Sep Tech.* 2015;6: 266.
97. Mottaleb MA et al. Use of LCMS and GC-MS Methods to Measure Emerging Contaminants Pharmaceutical and Personal Care Products (PPCPs) in Fish. *J Chromatogr Sep Tech.* 2015;6:267.
98. Matheis Ket al. Combining TLC Separation with MS Detection - A Revival of TLC. *J Glycomics Lipidomics.* 2015;5:e125.
99. Tyagi A et al. HPTLC-Densitometric and RP-HPLC Method Development and Validation for Determination of Salbutamol Sulphate, Bromhexine Hydrochloride and Etofylline in Tablet Dosage Forms. *Pharm Anal Acta.* 2015;6:350.
100. Shantabi L, Jagetia GC, Vabeiryureilai M, Lalrinzuali K (2014) Phytochemical Screening of Certain Medicinal Plants of Mizoram, India and their Folklore Use. *J Biodivers Biopros Dev* 1:136. <http://omicsgroup.org/journals/2169-0022/2169-0022.S1.014-005.pdf>
101. [http://omicsonline.org/2157-7463/2157-7463.S1.006\\_043.pdf](http://omicsonline.org/2157-7463/2157-7463.S1.006_043.pdf)
102. <http://omicsonline.org/2161-0398/2161-0398.S1.008-010.pdf>
103. <http://omicsonline.org/2161-0398/2161-0398.S1.006-061.pdf>

104. <http://omicsonline.org/2161-0398/2161-0398.S1.006-033.pdf>
105. <http://omicsonline.org/2161-0398/2161-0398.S1.008-034.pdf>
106. <http://omicsgroup.org/journals/2169-0022/2169-0022-S1.010-004.pdf>
107. <http://omicsonline.org/2155-9872/2155-9872.S1.01-003.pdf>
108. <http://omicsgroup.org/journals/2169-0022/2169-0022.S1.014-094.pdf>
109. AbMajid AH et al. Colony Genetic Organization and Breeding Pattern of Subterranean Termite (*Reticulitermesflavipes*) over the Three Field Seasons in Nebraska, U.S.A. *EntomolOrnitholHerpetol.* 2014;3:133.
110. Sattar A et al. Efficacy of Plant Extracts Against Subterranean Termites i.e., *Microtermesobesi* and *Odontotermeslokanandi* (Blattodea:Termitidae). *J BiodiversBiopros Dev.* 2014;1:122.
111. Li T et al. New Insights into Microbes in the Midgut of Termite *Coptotermesformosanus*. *J BioremedBiodeg.* 2014;5:220.
112. Igwe CU et al. Chemical Analysis of an Edible African Termite, *Macrotermesnigeriensis*; a Potential Antidote to Food Security Problem. *Biochem& Anal Biochem.* 2011;1:105.
113. Tariq MH et al. Development and Validation of a Simple, Accurate and Economical Method for the Analysis of Vancomycin in Human Serum Using Ultracentrifuge Protein Precipitation and UV Spectrophotometer. *J Anal Bioanal Tech.* 2011;6:239.
114. Takegami S et al. Application of <sup>19</sup>F NMR Spectroscopy Using a Novel  $\alpha$ -Tocopherol Derivative as a <sup>19</sup>F NMR Probe for a Pharmacokinetic Study of Lipid Nano-Emulsions in Mice. *Pharm Anal Acta.* 2015;6:339.
115. Xu C et al. <sup>1</sup>H NMR-based Plasma Metabolic Profiling of Dairy Cows with Type I and Type II Ketosis. *Pharm Anal Acta.* 015;6:328.
116. Chen Fu. Basic Principles and Applications of Solid-State NMR in Catalysis. *J ThermodynCatal.* 2014;5:e127.
117. Chairman M et al. Phytochemical Screening and Antimicrobial Activity of *Curcuma longa* Natural Dye. *General Med.* 2015;3:171.
118. Kohls S et al. Cardiac glycosides from Yellow Oleander (*Thevetia peruviana*) seeds. *Phytochemistry.* 2012;75:114-127.