Original Research Article

Evaluation of the effects of drying methods on phytochemicals in *Thevetia neriifolia* parts as a potential rodenticide

Abstract

Synthetic rodenticides are effective and rapid in controlling rats; however, they are toxic to non-target species including humans, as well as the environment. *Thevetia neriifolia* (Pers.) K. Schum different parts however, reportedly have toxic effects on rodents. Effects of fresh, air and sun-drying methods on phyto-constituents of flowers, leaves, root, seed and stem bark and its potentiality in the control of rodents were therefore, investigated in this study. Analyses showed the presence of free agyclones: Thevetin A (1.88mg/g) and B (1.64 mg/g), cardiac glycosides (1.49mg/g), alkaloid (1.36 %) and digitoxin (1.32 mg/g) in highest concentrations. Phenols ($6.90x10^{-2}$ mg/g), ($2.21x10^{-2}$mg/g) from flavonoids, Tannins ($1.13x10^{-2}$mg/g) and steroids ($6.70x10^{-3}$ mg/g) were in moderate concentrations while antraquinone ($0.70x10^{-3}$mg/g) were the lowest. Antraquinone was also not detected in flower part. Sun-dried parts had highest concentrations of Thevetin A (1.47 mg/g), cardiac glycosides (1.39 mg/g) and Thevetin B (1.27mg/g) followed by air–drying for cardiac glycoside (1.33mg/g) and Thevetin A. Fresh for Thevetins A 1.25mg/g, and B 1.10mg/g, and cardiac glycoside 0.93mg/g and tannins, respectively while antraquinone and terpenes were undetected. Higher phytochemicals content were in leaf followed by stem bark, then air-drying due to interaction of sun-drying methods and parts of plant. Free aglycones Thevetins A and B, cardiac glycosides, digitoxin, oleadrin, tannins, phenols and steroids in *Thevetia neriifolia* plant parts were not affected by different drying methods. Therefore, the relatively high cardiac glycosides and free aglycone in different parts of *Thevetia neriifolia* may be exploited for natural rodenticidal purpose.

Keywords: *Thevetia neriifolia*, Phytochemicals-concentration, Drying-methods, Cardiac glycosides, Free aglycones

Introduction

Thevetia neriifolia (Pers.), yellow oleander is a small tree which belongs to Apocynaceae family, generally used as ornamental plant (1). Details of botanical description (2) and medical uses (3, 4, 5, 6) of *Thevetia neriifolia* have been documented. Earlier reports of studies on plant parts

revealed that seeds of *T. peruviana* possesses insecticidal (7, 8), fungicidal (9, 10), bactericidal (9, 11) and rodenticidal (12) values.

In literature, (13, 14, 15, 16) all parts of *Thevetia neriifolia,* especially, seeds were reportedly toxic to vertebrates and rodents. However, properly soaked and appropriately processed seeds was edible and of high nutritional quality to broiler chickens (17, 18).

Although, synthetic chemicals are often used and are more effective in rapid control of rats, there have been documented accidents (19) due to consumption by non targeted organisms particularly, humans. Rodenticides specially designed to kill rodents, pose particular risk for accidental poisoning because rodents usually share human environments. Rodenticides are controversial due to secondary poisoning and their risks of exposure to humans, pets, non-target species, wildlife and environment (19). Also, there have been recent reports (20) of the likely resistance and resurgence of new breeds of pests due to perpetual use of synthetic chemicals as rodenticides with the attendant hazardous effects on food and environment. There is therefore the need for natural rodenticides that will be cheaper, readily available and not likely impact negatively on human food and environment (21).

Available reports on effects of drying methods on phytochemicals in *Thevetia peruviana* were mostly based on seeds (17, 18. 22) with dearth of information on its concentration on other parts of the plant. It was envisaged that *Thevetia neriifolia* plant parts in powdery form could serve as a baseline for a potential natural rodenticide in form of bait. The current study was therefore, aimed at ascertaining the relative presence and composition of phytochemicals in the different parts of *Thevetia neriifolia* (Pers) as will be affected by the different drying methods.

Materials and Methods

Experimental site: This study was undertaken at the Department of Crop Protection and Environmental Biology (CPEB), University of Ibadan, Ibadan, Nigeria. The Department is located within latitude 7^0 43"N and longitude 3^0 54"E at an altitude of 200m with annual rainfall between 1,250-1,500mm spanning eight months (March-October) with dry spell in August; annual average temperature of 21.3^0C and relative humidity of 70-80%.

Samples Sourcing: All plant parts: leaves, flowers, seeds, stem bark and roots of *Thevetia neriifolia* (Pers) were collected from Faculty of Education, University of Ibadan, Nigeria in the

month of March, 2013. Collection was by direct plucking and picking of those that had fallen off plants. The stem bark decoction and root cutting were done using a cutlass. The fruits were cracked to remove the hard pericarp and mesocarp.

Experimental design: The experiment was a '3x5' factorial arrangement (comprising three drying methods and five different plant parts) in a completely randomized design and replicated three times.

Preparation of Samples

The plucked and picked *Thevetia neriifolia* plant parts were divided into two for air-drying and sun-drying. Air-drying of plant parts was carried out at the Toxicology Research Laboratory, Crop Protection and Environmental Biology, University of Ibadan at the temperature of 27±2^{o}C and relative humidity of 60-70% for twenty days. The sun-dried plant parts were dried at the average temperature of 26 $\pm$ 4^{o}C and relative humidity of 84.5% for a period of 10 days. The dried seeds collected were cracked to remove the kernels. The air and sun-dried samples were separately milled using electric blender machine and were sieved with 2mm wire mesh to obtain fine granules (powder). They were weighed and packaged in sample bottles for analysis.

Quantitative determination of phytochemicals and cardiac glycosides in *T. neriifolia*

Method of Sample Extraction: Cold water extraction was used. Twenty eight grams each of finely ground plant part sample was dissolved in 140mls of distilled water in a 250mLs conical flask and covered with aluminium paper for 24 hours continuously shaken on a shaker after which it was filtered. The filtrate was concentrated on a water bath at 40^{0}C and labeled (23).

Alkaloid was determined quantitatively using the method of Henry (24). Flavonoid was determined according to Allen (25) as modified (26). Saponin was analyzed spectrophotometrically by method of Brunner (27). Tannin was determined quantitatively by the method of Sofowora (28). Quantitative analyses of phenols, terpenes, steroids and cardiac glycosides, thevetins A and B, digitoxins (free aglycones) were determined analytically (29). All determinations were in triplicates

Statistical Analyses

Data were subjected to three-way analyses of variance using the statistical package of SAS (30) and means were separated using Duncan multiple range test of the same software

Results:

The main effect of plant parts (flower, leaves, root, seed and stem) on phytochemical composition is presented in Table 1. Significant differences ($p<0.05$) were obtained in the values of the different phytochemicals in *Thevetia neriifolia* parts.

Leaf contained significantly higher ($p<0.05$) flavonoids (mg/g) ($2.21x10^{-3}$) compared to flower ($1.59x10^{-3}$), stem bark ($1.27x10^{-3}$), seed ($0.79x10^{-3}$) and root ($0.66x10^{-3}$). Alkaloid (%) was higher ($p>0.05$) in both the stem bark (1.36) and leaves (1.25) compared with root (0.83), seed (0.77) and flower (0.64). Saponin (mg/g) composition varied significantly with parts of the plant. Higher saponin (mg/g) (1.22) was in the leaves but lower (0.45) in flower. The stem bark contained higher antraquinone (mg/g) ($0.70x10^{-3}$) similar to $0.49x10^{-3}$ in root but significantly higher ($p<0.05$) than $0.20x10^{-3}$ in leaves and $0.17x10^{-3}$ in seed while anthraquinone was not detected in flower.

Tannins (mg/g) was higher in seed ($1.13x10^{-2}$), flower ($1.11x10^{-2}$) and stem bark ($1.02x10^{-2}$) compared with leaves ($0.70x10^{-2}$) and root ($0.49x10^{-2}$). The seed contained significantly higher levels ($p<0.05$) of phenols (mg/g) ($6.90\ x10^{-2}$) followed by stem bark ($3.29x10^{-2}$) while flower ($1.52x10^{-2}$), leaves ($1.52x10^{-2}$) and root ($0.91x10^{-2}$) contained relatively lower values. Terpene (mg/g) was higher in flower ($6.31x10^{-3}$) and seed ($3.46\ x10^{-3}$) compared with leaves ($1.58x10^{-3}$), stem bark ($0.87x10^{-3}$) and root ($0.42x10^{-3}$). The steroids (mg/g) in flower ($6.70x10^{-3}$) was significantly higher ($p<0.05$) compared with leaves ($2.18x10^{-3}$), root ($0.69x10^{-3}$), seed ($3.01x10^{-3}$) and stem bark ($0.97x10^{-3}$).

Cardiac glycosides (mg/g) were higher in leaves (1.49) while lower value of 0.97 was obtained from the root. The leaves also contained significantly higher ($p<0.05$) cardenolide (mg/g) (1.22) when compared with other plant parts. Both stem bark and leaves contained significantly higher ($p<0.05$) chalcones (mg/g) with respective values of $7.77x10^{-3}$ and $5.63x10^{-3}$ while flower ($1.88x10^{-3}$), root ($1.18x10^{-3}$) and seed ($2.59x10^{-3}$) contained similar lower values. Stem bark had

Table 1: Main effect of different plant parts of *Thevetia neriifolia* on phytochemicals composition

Parameters	Flower	Leaves	Root	Seed	Stem Bark	SEM
Flavonoids ($x10^{-3}$)	1.59^{ab}	2.21^{a}	0.66^{c}	0.79^{c}	1.27^{bc}	0.14
Alkaloid	0.64^{b}	1.25^{a}	0.83^{b}	0.77^{b}	1.36^{a}	0.07
Saponin	0.45^{e}	1.22^{a}	0.81^{c}	0.68^{d}	0.93^{b}	0.04
Antrquinones ($x10^{-3}$)	-	0.20^{b}	0.49^{a}	0.17^{b}	0.70^{a}	0.07
Tanins ($x10^{-2}$)	1.11^{a}	0.70^{bc}	0.49^{c}	1.13^{a}	1.02^{ab}	0.07
Phenols ($x10^{-2}$)	1.52^{b}	1.52^{b}	0.91^{b}	6.90^{a}	3.29^{ab}	0.76
Terpene ($x10^{-3}$)	6.31^{a}	1.58^{b}	0.42^{b}	3.46^{ab}	0.87^{b}	0.70
Steroids ($x10^{-3}$)	6.70^{a}	2.18^{b}	0.69^{b}	3.01^{b}	0.97^{b}	0.68
Cardiac glycosides	1.13^{c}	1.49^{a}	0.97^{d}	1.11^{c}	1.37^{b}	0.04
Cardenoides	0.91^{c}	1.22^{a}	0.84^{cd}	0.81^{d}	1.10^{b}	0.04
Chalcoones ($x10^{-3}$)	1.88^{b}	5.63^{ab}	1.18^{b}	2.59^{b}	7.77^{a}	0.81
Phlobatin ($x10^{-3}$)	1.72^{b}	1.44^{b}	0.86^{b}	1.43^{b}	8.56^{a}	0.76
Thevetin_a	0.95^{c}	1.88^{a}	0.88^{c}	1.46^{b}	1.59^{b}	0.06
Thevetin_b	0.86^{d}	1.64^{a}	0.71^{e}	1.19^{c}	1.42^{b}	0.06
Digitoxin	0.70^{d}	1.32^{a}	0.73^{d}	0.79^{c}	1.05^{b}	0.04
Oleanderin	0.85^{b}	1.24^{a}	0.67^{c}	0.88^{b}	0.87^{b}	0.03

$^{a, b, c, d, e, f}$ Means along the same row with different superscripts are significantly ($p<0.05$) different

C.glycos = Cardiac glycoside, Antrqin= Anthraquinine, Flavo= Flavonoids

Carden= Cardenolides, Chalco=Chalcones

SEM =Standard Error Mean

significantly ($p<0.05$) higher phlobatanin (mg/g) ($8.56x10^{-3}$) compared with other plant parts which contained similar values ($p<0.05$) ranging from 0.86 $x10^{-3}$ in root to $1.72x10^{-3}$ in flower. Thevetin A (mg/g) was significantly higher ($p<0.05$) in leaves (1.88). Similar levels of Thevetin A (mg/g) were obtained from stem bark (1.59) and seed (1.46) while flower (0.95) and root (0.86) contained lower levels. The leaves had significantly higher ($p<0.05$) (1.64) Thevetin B (mg/g), followed by 1.42 in the stem bark which differed from 1.19 in seed, 0.86 in flower and much lower (0.71) in root. Digitoxin (mg/g) was higher in leaves (1.32) than stem bark (1.05) which also differed from the values of 0.79, 0.70 and 0.73 in seed, flower and root, respectively. Oleander (mg/g) was higher in leaves (1.24) and lower in root (0.67) while flower, seed and stem bark contained similar ($p>0.05$) values of 0.85, 0.88 and 0.87, respectively.

Main effect of drying methods on phytochemicals in *Thevetia neriifolia* plant parts is shown in Table 2. Results revealed that drying methods had no significant effect ($p>0.05$) on flavonoids (mg/g), phenols (mg/g), terpenes (mg/g) and chalcones (mg/g) composition of test plant. Effect of drying methods was significant ($p<0.05$) on alkaloid (%) composition of *Thevetia neriifolia*. Air-drying lowered alkaloid (%) significantly ($p<0.05$) from $1.07x10^{-3}$ in fresh sample and 1.18 x 10^{-3} in sun dried sample to $0.65x10^{-3}$. Sun-dried samples of *Thevetia* contained significantly ($p<0.05$) higher saponin (mg/g) (0.89) levels compared with 0.78 and 0.79 in both fresh and air dried samples, respectively. Sun-dried samples contained significantly higher ($p<0.05$) antraquinone (mg/g) ($0.67x10^{-3}$) compared with ($0.27x10^{-3}$) in air-dried samples, while antraquinone (mg/g) was not detected in fresh samples. Air-dried samples had significantly higher ($p<0.05$) tannins (mg/g) ($1.08x10^{-2}$) compared with sun-dried and fresh *Thevetia* samples. Sun-dried and fresh samples of *Thevetia* had similar values of tannins (mg/g) ($0.80x10^{-2}$ and $0.79x10^{-2}$), respectively. Phenols (mg/g) and terpenes (mg/g) values were not significantly ($p>0.05$) affected by drying methods. Higher value of steroids (mg/g) ($5.95x10^{-3}$) was obtained from sun-dried parts.

Lower value of steroid (mg/g) ($0.92x10^{-3}$) was obtained from air-dried and fresh samples. Similarly, higher values of cardiac glycosides (mg/g) (1.39 and 1.33) were respectively obtained from both sun-dried and air-dried samples. Fresh samples had the lower value (0.93) (mg/g) of

cardiac glycoside. Effect of drying methods on chalcones (mg/g) contents was not significantly different ($p>0.05$) among all treatments. Phlobatanin (mg/g) ($5.49x10^{-3}$) was higher in the air-dried samples. Both sun-dried and fresh plant parts contained similar ($p>0.05$) values of phlobatanin (mg/g) ($1.14x10^{-3}$). Sun-drying method left significantly higher ($p<0.05$) residual

Table 2: Main effect of drying methods on phytochemicals in *Thevetia neriifolia* plant parts

Parameters	Air-drying	Fresh	Sun-drying	SEM
Flavonoids ($x10^{-3}$)	1.32	1.03	1.55	0.14
Alkaloid	0.65^{b}	1.07^{a}	1.18^{a}	0.07
Saponin	0.78^{b}	0.79^{b}	0.89^{a}	0.04
Antraquinones ($x10^{-3}$)	0.27^{b}	-	0.67^{a}	0.07
Tanins ($x10^{-2}$)	1.08^{a}	0.79^{b}	0.80^{b}	0.07
Phenols ($x10^{-2}$)	1.40	2.47	4.62	0.76
Terpene ($x10^{-3}$)	2.95	-	4.63	0.70
Steroids ($x10^{-3}$)	1.25^{b}	0.92^{b}	5.95^{a}	0.68
Cardiac_glycosides	1.33^{a}	0.93^{b}	1.39^{a}	0.04
Cardenolides	1.07^{a}	0.74^{b}	1.12^{a}	0.04
Chalcoones ($x10^{-3}$)	4.18	5.39	1.86	0.81
Phlobatin ($x10^{-3}$)	5.49^{a}	1.14^{b}	1.78^{b}	0.76
Thevetin a	1.33^{b}	1.25^{b}	1.47^{a}	0.06
Thevetin_b	1.13^{b}	1.10^{b}	1.27^{a}	0.06
Digitoxin	0.94^{a}	0.85^{b}	0.98^{a}	0.04
Oleanderin	0.93^{a}	0.79^{b}	0.99^{a}	0.03

$^{a, b, c, d, e, f}$ Means along the same row with different superscripts are significantly ($p<0.05$) different

value of Thevetin A (mg/g) (1.47) compared with other drying methods. Both air-dried and fresh samples had similar values while the fresh had the lower value of Thevetin A (mg/g) (1.25).

Significantly higher value of Thevetin B (mg/g) (1.27) was obtained from sun-dried samples while there was lower value (1.10) from fresh sample. Similarly, higher digitoxin (mg/g) were obtained from sun-dried and fresh samples (0.98 and 0.94), respectively. However, lower value (0.85) was obtained from fresh samples. Oleander (mg/g) was higher in sun-dried (0.99) and air-dried (0.93) of *Thevetia* than in fresh plant (0.79).

Effects of interactions of plant parts and methods of drying *Thevetia* are presented in Table 3. The sun ($3.27x10^{-3}$) and air-dried $3.07x10^{-3}$) leaf parts had significantly higher ($p<0.05$) flavonoids (mg/g) compared to other plant parts and drying methods. Fresh flower part also had significantly higher ($p<0.05$) ($2.23x10^{-3}$) but similar ($p>0.05$) to sun-dried stem bark which had $1.70x10^{-3}$. Fresh leaf contained lower flavonoid (mg/g) ($0.28x10^{-3}$) Alkaloid (%) was higher in sun-dried stem bark (1.52) plant part compared to other parts. Higher alkaloid (%) value of 1.43 was obtained from fresh stem bark. Alkaloid (%) contents of leaf and root parts when sun dried or air-dried were not significantly different ($p>0.05$). Also, no significant difference ($p>0.05$) was observed in alkaloid content of fresh and sun-dried flower.

Saponin (mg/g) was higher in sun-dried (1.26) and fresh (1.19) leaves. Higher saponin (mg/g) value of 1.15 was also obtained from sun-dried stem bark. Saponin (mg/g) was lower in fresh flower part (0.41).Antraquinone (mg/g) was significantly higher in sun-dried stem bark ($1.33x10^{-3}$). There were no significant differences ($p>0.05$) in the values obtained from the sun dried root and air-dried stem bark parts. There were no significant differences ($p>0.05$) among the leaf, root and seed parts subjected to sun-drying air-drying and sun-drying methods, respectively. However, antraquinone (mg/g) was not detected in fresh and air dried leaf, root, seed, fresh stem bark and in flower samples subjected to all methods of drying.

Air-dried stem bark had higher tannins (mg/g) ($1.73x10^{-2}$) followed by sundried seed ($1.30x10^{-2}$). No significant differences were found in levels of tannins (mg/g) in air and sun-dried flowers, air-dried seed and fresh stem bark. There were no differences among obtained contents of tannins (mg/g) due to plant parts and the drying methods of flower (fresh), leaf and root (sun, air-

191 drying and fresh), seed (fresh) and stem bark (sun-drying). Phenol (mg/g) was higher in sun-
192 dried seed part (18.33 $x10^{-2}$). There was no significant difference ($p>0.05$) in phenol (mg/g)

193 **Table 3: Interaction effects of plant parts and methods of drying of *Thevetia neriifolia***

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Parameters	Drying Method	Flower	Leaves	Root	Seed	Stem Bark	SEM
Flavonoids ($x10^{-3}$)	Air-drying	1.13^{cdef}	3.07^{a}	0.93^{cdefg}	0.59^{efg}	0.87^{defg}	
	Fresh	2.23^{b}	0.28^{g}	0.60^{efg}	0.80^{defg}	1.23^{cde}	
	Sun-drying	1.40^{cd}	3.27^{a}	0.44^{fg}	0.97^{cdefg}	1.70^{bc}	0.15
Alkaloid	Air-drying	0.00j	1.25^{cd}	0.88h	0.00j	1.12^{ef}	
	Fresh	0.93^{gh}	1.21^{d}	0.71i	1.10^{f}	1.43^{b}	
	Sun-drying	0.99g	1.30^{c}	0.90h	1.19^{de}	1.52^{a}	0.01
Saponin	Air-drying	0.46g	1.22^{abf}	0.84^{d}	0.69^{ef}	0.69^{ef}	
	Fresh	0.41g	1.19^{ab}	0.74^{e}	0.64^{f}	0.96^{c}	
	Sun-drying	0.49g	1.26^{a}	0.86^{d}	0.70^{ef}	1.15^{b}	0.02
Antraquinones ($x10^{-3}$)	Air-drying	-	-	0.57^{bc}	-	0.77^{ab}	
	Fresh	-	-	-	-	-	
	Sun-drying	-	0.60^{bc}	0.90^{ab}	0.50^{bc}	1.33^{a}	0.13
Tanins ($x10^{-2}$)	Air-drying	1.23^{abc}	0.70^{bcdef}	0.50^{def}	1.23^{abc}	1.73^{a}	
	Fresh	0.97^{bcde}	0.63^{cdef}	0.37^{ef}	0.87^{bcde}	1.13^{abcd}	
	Sun-drying	1.13^{abcd}	0.77^{bcdef}	0.60^{cdef}	1.30^{ab}	0.19^{f}	0.12
Phenols ($x10^{-2}$)	Air-drying	1.77^{b}	1.57^{b}	0.40^{b}	1.20^{b}	2.07^{b}	
	Fresh	1.60^{b}	1.03^{b}	0.97^{b}	1.17^{b}	7.57^{b}	
	Sun-drying	1.19^{b}	1.97^{b}	1.37^{b}	18.33^{a}	0.23^{b}	1.61
Terpene ($x10^{-3}$)	Air-drying	1.60^{a}	2.10^{c}	0.50^{c}	9.33^{b}	1.23^{c}	
	Fresh	-	-	-	-	-	
	Sun-drying	17.3^{a}	2.63^{c}	0.77^{c}	1.03^{c}	1.37^{c}	0.71
Steroids ($x10^{-3}$)	Air-drying	1.60^{c}	2.13^{c}	0.77^{c}	0.63^{c}	1.13^{c}	
	Fresh	1.17^{c}	1.87^{c}	0.43^{c}	0.70^{c}	0.43^{c}	
	Sun-drying	17.3^{a}	2.53^{c}	0.87^{c}	7.70^{b}	1.33^{c}	0.98
Cardiac glycosides	Air-drying	1.16^{f}	1.69^{a}	1.02g	1.20^{ef}	1.56^{c}	
	Fresh	1.02g	1.05g	0.80i	0.86i	0.92h	
	Sun-	1.22^{ed}	1.74^{a}	1.07g	1.28^{d}	1.62^{b}	0.01

	drying						
Cardenolides	Air-drying	0.99^{de}	1.34^{ab}	0.92^{ef}	0.86^{f}	1.24^{c}	
	Fresh	0.72g	0.90^{ef}	0.66g	0.70g	0.74g	
	Sun-drying	1.02^{d}	1.43^{a}	0.95^{def}	0.86^{f}	1.32^{bc}	0.02
Chalcones (x10^{-3})	Air-drying	1.87^{b}	14.00^{a}	1.20^{b}	1.60^{b}	2.23^{b}	
	Fresh	1.70^{b}	1.30^{b}	0.90^{b}	4.37^{b}	18.67^{a}	
	Sun-drying	2.07^{b}	1.60^{b}	1.43^{b}	1.80^{b}	2.40^{b}	1.31
Phlobatin (x10^{-3})	Air-drying	1.77^{bcde}	1.63^{bcdef}	0.87^{efg}	1.50^{cdefg}	21.67^{a}	
	Fresh	1.37^{cedfg}	0.80^{fg}	0.67g	1.30^{cdefg}	1.57^{bcdefg}	
	Sun-drying	2.03^{bc}	1.90^{bcd}	1.03^{defg}	1.50^{cdefg}	2.43^{b}	0.17
Thevetin_a	Air-drying	0.93^{d}	1.74^{b}	0.91^{d}	1.53^{bc}	1.58^{bc}	
	Fresh	0.90^{d}	1.71^{b}	0.82^{d}	1.24^{cd}	1.56^{bc}	
	Sun-drying	1.02^{d}	2.19^{a}	0.92^{d}	1.59^{bc}	1.62^{bc}	0.08
Thevetin_b	Air-drying	0.82^{efg}	1.64^{ab}	0.70g	1.15^{def}	1.32^{abcd}	
	Fresh	0.72^{fg}	1.62^{abc}	0.66g	1.20^{cde}	1.28^{abcd}	
	Sun-drying	1.04^{defg}	1.66^{a}	0.76^{fg}	1.22^{bcde}	1.66^{a}	0.08
Digitoxin	Air-drying	0.70^{fg}	1.32^{a}	0.72^{fg}	0.88^{e}	1.07^{bc}	
	Fresh	0.66^{gh}	1.29^{a}	0.69^{fgh}	0.61^{h}	0.99^{cd}	
	Sun-drying	0.75^{f}	1.35^{a}	0.77^{f}	0.90^{ed}	1.10^{b}	0.02
Oleanderin	Air-drying	0.83^{ef}	1.24^{ab}	0.68^{hi}	1.03^{c}	0.87^{e}	
	Fresh	0.76^{fg}	1.20^{b}	0.62^{i}	0.56^{j}	0.79^{f}	
	Sun-drying	0.96^{d}	1.27^{a}	0.72^{gh}	1.05^{c}	0.95^{d}	0.01

a, b, c, d, e, f, ….Means along the same column within each subgroup and along the same row with different superscripts are significantly ($p<0.05$) different

contents obtained in other plant parts due to drying methods. Terpene (mg/g) was higher in sun-dried flower (17.3 x10^{-3}) compared with other dried and fresh parts while sun-dried seed (1.03x10^{-3}) was lower. Air-dried seed contained appreciable level of terpene (mg/g) (9.33x10^{-3}). Other plant parts had similar terpenes (mg/g) due to drying methods. Higher level of steroid (mg/g) (17.3x10^{-3}) was obtained from sun-dried flower, followed by sun-dried seed (7.70x10^{-3}), while other plant parts did not have significantly ($p>0.05$) varied levels of terpene (mg/g) due to drying methods.

Sun-dried and air-dried leaf contained significantly higher ($p<0.05$) cardiac glycoside (mg/g) (1.74 and1.69, respectively) followed by the sun-dried and air-dried stem bark with the values of 1.62 and 1.56, respectively. Significant differences ($p>0.05$) were not observed in the cardiac glycoside (mg/g) contents of air-dried flower and seed, similar trend was observed in sun-dried flower and seed. Also, no differences were found in fresh flower, leaf and sun-dried as well as air-dried root. Least value of cardiac glycoside (mg/g) (0.80) was found in the fresh root. Sun and air-dried leaf parts higher cardenolide (mg/g) (1.43 and 1.34, respectively). There were no observed significant differences ($p>0.05$) in contents of cardenolide (mg/g) in air-dried flower and root, air-dried root and seed, fresh leaf and flower, fresh and sun-dried root and seed. Fresh root contained least contents of cardenolide (mg/g) (0.66). Chalcone (mg/g) was higher in fresh and air dried stem bark with the respective values of $18.67x10^{-3}$ and $14.00x10^{-3}$. Effects of interaction of plant parts and methods of drying on values from other plant parts were not significantly different ($p>0.05$).

Significant differences were observed in the contents of phlobatanin (mg/g) with higher value in air-dried stem bark ($21.67\ x10^{-3}$). Significant differences ($p>0.05$) were not observed in effects of interaction of plant parts and the drying methods on phlobatanin (mg/g) in other parts were not significantly different ($p>0.05$). Least phlobatanin (mg/g) level ($0.67\ x10^{-3}$) was obtained from fresh root. Significantly higher ($p<0.05$) Thevetin A (mg/g) was obtained from sun-dried leaf (2.19). The fresh and the air-dried leaf parts had higher Thevetin A (mg/g) with the values of 1.74 and 1.71, respectively. Effects of interaction of drying methods and parts of plant were not significant ($p>0.05$) on the values of Thevetin A (mg/g) in flower, root and stem bark.

Sun-dried leaf and stem bark had significantly ($p<0.05$) higher Thevetin B (mg/g) (1.66 and 1.66, respectively) while values obtained other parts with different drying methods were similar ($p>0.05$). Effects of interaction of different plant parts and drying methods on digitoxin (mg/g) was significant ($p<0.05$). The digitoxin (mg/g) contents of sundried (1.35), air-dried (1.32) and fresh (1.29) leaves were higher compared to other plant parts. Least digitoxin (mg/g) was obtained from fresh seed (0.61). The interactive effects of plant parts and drying methods on digitoxin (mg/g) in all parts of plants were significantly different ($p<0.05$). Sun-dried and air-dried leaf had significantly higher ($p<0.05$) oleandrin (mg/g) (1.27 and 1.24, respectively).

Higher oleandrin (mg/g) content was also obtained from fresh leaf (1.20) while least oleandrin (mg/g) was obtained from fresh seed and (0.50).

Interaction Results on *Thevetia neriifolia*

Effect of interaction of drying methods and parts of *Thevetia neriifolia* on alkaloid was significantly higher ($p<0.05$) on sun-dried stem bark (1.52) compared with fresh stem bark (1.43). Lower ($p<0.05$) values were obtained from air-dried flower and seed (0.00), respectively. Significantly higher ($p<0.05$) saponins was in sun-dried leaf (1.26), followed by air-dried (1.22) and fresh (1.19), then, sun-dried stem bark (1.15). The air-dried, sun-dried and fresh flower contained lower ($p<0.05$) saponins (0.46).

Effect of interaction of drying methods and parts of *T. neriifolia* was significantly higher ($p<0.05$) on cardiac glycoside in sun-dried and air-dried leaves (1.74 and 1.69), respectively. This was followed by the stem bark (1.62) while significantly lower ($p<0.05$) cardiac glycosides and was in fresh seed (0.86) and root (0.80).

Interactive effects of drying methods and parts of *T. neriifolia* on Thevetin A content was significantly higher ($p<0.05$) in sun-dried leaf (2.19), followed by air-dried (1.74) and fresh leaves (1.71). Thevetin A was lower ($p<0.05$) in air-dried, sun-dried and fresh flower and root.

Effect of interaction methods of drying and parts of *T. neriifolia* on Thevetin B content was significantly higher ($p<0.05$) in the sun-dried, air-dried and fresh leaves (1.66, 1.64 and 1.62, respectively) and stem bark (1.66). Lower Thevetin B ($p<0.05$) was in the sun-dried, air-dried and fresh root (0.76, 0.70 and 0.66, respectively).

Digitoxin was significantly higher ($p<0.05$) in the sun-dried, air-dried and fresh leaf due to interactions of drying methods and parts of *T. neriifolia* (1.35, 1.32 and 1.29, respectively). Lower ($p<0.05$) digitoxin was in the fresh root (0.69) and seed (0.61).

Effect of the interaction of drying methods and parts of *T. neriifolia* was higher ($p<0.05$) for oleander in the sun-dried, air-dried and fresh, leaf (1.27, 1.24 and 1.20, respectively). Lower ($p<0.05$) oleander was found in the fresh seed (0.56).

Discussion

The screening of *Thevetia neriifolia* plant parts (flower, leaf, root, seed and stem bark) for phytochemicals revealed the presence of some active ingredients in all parts of plant. Thevetins A and B, cardiac glycosides, digitoxin, oleander, alkaloid and saponin were the main abundant active constituents. Tannins, flavonoids, phenols and steroids were present moderately while antraquinone was absent in flower parts. Similar observation was obtained on the effects of interactive effects of plant parts and the different drying methods (air-drying, fresh and sun-drying). This observation agreed with the earlier reports of Gata-Goncalves *et al.* (8) as well as that of Essiett and Udofa (39) that phytochemicals were present in all the parts (leaves, stems and flowers) of *T. neriifolia* with moderate tannins and high cardiac glycosides in leaf part.

These active phytochemicals were most abundant in the leaf, followed by the stem bark. The leaf part contained the greatest oleandrin and very high digitoxin concentrations which may explain the reasons for the toxicity of *T. neriifolia* (31, 32). Other studies (31, 32) also concluded that the leaf part contained the greatest oleandrin and very high digitoxin concentrations. Studies on vertebrates (33) showed that the leaf and stem bark of *T. neriifolia* possesses very effective piscicidal activity. The stem bark was also reported to have toxic effects on rats by reducing the weight of the reproductive organs, a fall in total protein and glycogen of the sperms hence reduction in fertility (34).

The seed of *T. neriifolia* in this study was found to contain low active constituents of cardiac glycoside and cardenolides, which was contrary to reports from earlier studies (5, 12, 15; 16, 35, 36). This may be due to the time of flowering of the plant, in plant species differences and contents of fresh and dried parts (37, 38). The relatively high concentrations of tannins and low concentrations of cardiac glycosides and other active ingredients in the flower conformed to the findings of Essiett and Udofa (39) who reported moderate tannins and trace concentration of cardiac glycoside in the flower part of *T. neriifolia*. The concentration of phytochemicals in root part was lowest in this study. This conformed to the reported smaller concentrations of oleandrin in the root when compared with other plant parts such as the leaves, stems and flowers (37). Conversely, Karawya *et al*. (37) observed that the roots contained higher concentrations of cardenolides.

The high alkanoids concentrations obtained in all parts of the plant with all drying methods indicated the potentialities of these plant parts as poison agent (40). It was also noted (41) that alkaloids caused developmental toxicity in rodents. Highly concentrated saponins found in all the *T. neriifolia* plant parts under the drying methods conformed to the work of Varsha (2). Saponin which was also found in *Thevetia* was noted for its extensive usage as detergent globally and was highly toxic when injected into the blood stream due to its reaction with enzymes (26). Also, saponins ingestion has been known to result in lysis of blood cells, haemolysis, carcinogenicity, neurotoxicity in rats and humans (42). Tannins concentration was higher in the leaves, flower, roots, seeds and stem bark of *T. neriifolia* when fresh, air-dried, and sun-dried thereby indicating starch and xanthoproteins in accordance with Varsha (2) and a damage to the livers of rats when ingested (43).

Anthraquine, a naturally occurring aromatic organic compound found in some plants and micro organisms is known to contribute to the colouring pigment. It is used for the manufacture of dyes commercially (44) and was present in very low concentrations. This compound was not detected in the flower of *T. neriifolia* in this study contrary to the report of Essiett and Udofa (39) that anthraquinone was detected in the flower of *T. peruviana.* However, anthraquine was detectable in the leaf and stem bark of *T. neriifolia* as was earlier documented (39). Though, both anthraquinone and terpene were undetectable in fresh samples but were present in sun-dried samples. This may be attributed to the concentration of active ingredients during drying.

This study showed that tannins, phenols, steroids, Thevetin A and B, digitoxin, cardenolides, cardiac glycosides and free aglycones were not affected by different drying methods in all the parts of *Thevetia neriifolia* plant. This corroborated earlier reports (45, 46, and 47) that drying or heating does not have effect on cardenolides, cardiac glycosides, Thevetin A and B though; sun-drying could cause concentration of active ingredients due to evaporation of water. Also, Sangodare *et al*. (48) reported that the components of this plant were not easily affected by heat because they produce gastric and cardio-toxic effects and may account for the potential use of this plant in the pest control (bio-pesticides).

Cardiac glycosides, cardenolides, Thevetin A and B (cerebroside) and free aglycones abundance in all parts of *T. neriifolia* irrespective of the drying methods employed in this study conformed

to the documented reports (46, 49) that these natural poisons made the plant to be toxic to vertebrates. Other workers (35, 50, 51, 52) also noted that the main active *Thevetia* phytoconstituent was cardiac glycosides which may also include the free aglycones such as Thevetin A and B, digitoxin as well as oleandrin.

Cardiac glycosides were reported to be toxic to rats as they damage their livers and hearts (54). Glycosides are also known to inhibit the transmembrane by binding to an extracellular portion of the Na^+/K^+ ATPases (sodium-potassium adenosine triphosphate enzyme) and cause increased intracellular calcium concentrations (32). The thevetins and other free aglycones of cardiac glycosides are also known to have toxic effects on the hearts (34), heart muscles, blood pressure elevation and heart irregularities. They also cause intestinal peristalsis, increased salivation and pupil contraction. The toxins (thevetin A, B and cardiac glycosides) have been reported to inhibit spermatogenesis in rats (34).

Conclusion

Drying of Thevetia neriifolia plant parts using different methods led to more concentration of inherent phytochemicals mostly in the leaf followed by stem bark. Cardiac glycosides, thevetin A and B, digitoxin and oleandrin concetrations in plant parts were not reduced by any of the drying methods. Therefore, any part of *Thevetia neriifolia* could be processed with appropriate drying methods (sun and air-drying) and be compounded to powder as natural bait (rodenticide) to control rats and other rodents.

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