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Nutritional Evaluation of Milk Bush (*Thevetia neriifolia*) Seed Oil in Wistar Albino Rats

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Abstract

The nutritional value of milk bush Thevetia neriifolia *seed oil was carried out in Wistar albino rats, 5%, 10% and 15% of the seed oil were administered to Wistar rats for 42 days (6 weeks) and various parameters like food intake, body weight changes, biochemical parameters like serum creatinine, liver and kidney catalase activity, superoxide dismutase (SOD) activity, lipid peroxidation, liver and heart cholesterol and triglycerides were determined. There was a significant decrease in liver cholesterol synthesis and triglycerides. On the other hand, there was a significant increase in kidney SOD catalase activity and a non-significant increase in liver SOD when the test rats were compared with the control group. A significant decrease in body weight and feed intake were observed when rats fed with 10% and 15% test diet were compared with the control. Finally, there was no significant change in liver and kidney catalase activity when test animals were compared with the control. The physicochemical studies of the seed oil showed the presence of 2.115 acid value, 70.125 mgKOH/g saponification value and 4.145 iodine value. Therefore,* Thevetia neriifolia *meets the nutritional requirements and if detoxified could be recommended for consumption.*

Keywords*: Nutritional evaluation, milk bush seed oil, Wistar albino rats, physicochemical properties.*

Introduction

Seeds have nutritive and caloric value, which make them necessary in diets. They are also good sources of edible oils and fats (Odeomelam 2005). Oil seeds are major sources of vegetable proteins and oils for human and animal nutrition. Apart from the domestic use of oils and fats as cooking oils, they also find wide application as sources of oleochemicals (Morrisonm *et al*. 1995). In Nigeria, the major sources of edible oils are peanut (*Arachis hypogea*) and oil palm (*Eloesis guinenis*). These oils are used mainly as cooking oils, they are also used for the production of soap, margarine, and cosmetics (Ong *et al*. 1995). With increasing demand, which has led to importation of cooking oils, there is need to source for local oil-bearing seeds which can be used in production of oils, both for consumption and industrial application. The production of palm oil is labour and capital intensive (Ong *et al*. 1995), hence the need to source for other local materials that will not require large amount of labour and capital. In Nigeria, notable among the inedible lesser known oil seeds are castor, *Jatropha curcas*, *Jatropha gossipifolia* and *Thevetia neriifolia* (Akintayo 2004).

Thevetia neriifolia

Thevetia plant is a dicotyledon which belongs to Aponaceae family. It is a composite evergreen shrub, which is found to have a milky sap. It is native to West Indians, Mexico and Brazil. It is known as yellow oleander (Nerium), gum bush, bush milk, exile tree in India, cabalonga in Puerto Rico, Olomi ojo by Yurubas in Nigeria. The plant is a shrub, reaching a height of 3.0-3.9 meters. The plant is perennial, the leaves are linear, narrow sword-like and green. The fruit when unripe is hard and green but gradually turns black as it

ripens. The fruits have varying masses (2-6.1 g) which are dispersed by man and propagated by seed or stem. The seed contains large quantity of oil about 57-63% oil on dry matter basis as reported by Lippold (1980).

The *Thevetia neriifolia* seed oil, when not purified, could be poisonous (Lippold 1980; Ibiyemi and Faloye 1990), however, after purification consists mainly of oleic acid (60%), palmitic acid (16%), stearic acid (11%), linolenic acid (7%) and linoleic acid (5%). The oil can be detoxified and could serve as vegetable oil for domestic use (Lippold 1980).

According to Ibiyemi *et al*. (2002), in spite of the toxicity of the plant, it has found useful application in several spheres of life. Its latex is used as an analgesic for tooth ache, the wood could be used as axe handle. The seed is 60-64% oil and 40-45% protein. The physical properties of the oil, particularly its saponification value and unsaponification matter, show that it can be used by commercial soap making industries.

Research on nutritional evaluation of *Thevetia neriifolia* seed oil is rare. This has prompted the study, so to evaluate its nutrition suitability.

Materials and Methods

Source and Preparation

The seed specimens for the study were collected at the front of the Department of Biochemistry, University of Ibadan, Ibadan, Oyo State, Nigeria, and identified at the herbarium of the Department of Botany, University of Ibadan. The air-dried seeds were coarsely powdered and subjected to hot hexane extraction for 6 hours in the laboratory to get the oil.

Physicochemical Properties

The physicochemical analysis was carried out according to the official methods of analysis of AOAC (1990). The physico-chemical properties determined included acid value, saponification value and iodine value.

Animals Used

Thirty male albino rats (Wistar strain) weighing between 70 g and 80 g were obtained in the Department of Physiology, University of Ibadan. On arrival, the rats were transferred and allowed to acclimatize for 7 (seven) days, being maintained on the standard normal diet with water *ad libium* in the animal house of the Department of Biochemistry under normal room temperature before the commencement of the experiment.

Experimental Design

The animals were distributed randomly according to their weight into four different groups. Each group had six animals. The control group received diet with normal feed, group A compounded diet with 5% of the milk bush seed oil, group B compounded diet with 10% of the milk bush seed oil, and group C compounded diet with 15% of milk bush seed oil.

Biochemical Analysis

Serum creatinine was estimated by the method of Bartels *et al.* (1972) while Catalase activity was determined according to the method of Sinha (1972). The level of SOD activity was determined by the method of Misra and Fridovich (1992). Lipid peroxidation was determined by measuring the formation of thiobarbituric acid reactive substances (TBARS) according to the method of Varshney and Kale (1990). Protein determination was carried out by means of the Biuret method, potassium iodine was added to the reagent to prevent precipitation of Cu^{2+} ions. Total serum cholesterol was determined according to the method of Allain *et al*. (1974). Triglyceride concentration was determined according to the principle described by Tietz (1990).

Results and Discussion

The result of proximate analysis of percentage of oil, as well as physicochemical properties, is shown in Table 1. The seed contains about 58.03% oil on dry matter basis, which agrees with Lippold (1980) but is lower than the value reported by Ibiyemi and Faloye (1990). This implies that the percentage of oil is very high, making it a good source of oil. The saponification value is 70.125 mgKOH/g. This is below the range of (188-196) for most

oils of plants origin (Pearson 1976). The low saponification value indicates that the oil has larger molecular weight than the common oils. This may be due to the presence of higher fatty acids. The low saponification value suggests that the oil can be used for candle and soap production and as chemical feedstock for lubricants (Shiina *et al*. 1986). The low acidic value indicates that the oil could be a good source of cooking oil without causing any harmful effect on the consumers.

The result of weight of liver and heart, as stated in Table 2, shows non-significant, this means that these organs were not affected. A significant decrease in body weight and feed intake was observed when rats fed with 10% and 15% test diet were compared with the control group. This could be that the feed was not palatable, which could be good for consumption after detoxification. There was no significant change in serum creatinine when test rats were compared with the control group as shown in Table 4. Since the rate of production of creatinine is constant, elevation of serum creatinine is indicative of under-excretion, suggesting kidney impairment, therefore, the result showed that there was no damage to kidney.

The result for liver lipids cholesterol in Table 5 showed significant decrease when 10% and 15% of rats fed with the seed oil were compared with the control. In heart lipid cholesterol, the result showed significant slight decrease when rats fed with 15% of the oil were compared with the control group. There was non-significant decrease in liver triglycerides when rats fed with test diet were compared with the control group. Furthermore, in heart triglycerides, there was a slight decrease when rats fed with 10% of the seed oil were compared with the control group as shown in Table 6. In Table 7, there was mild increase in SOD activity in rats fed with the seed oil when compared with the control group. SOD and catalase play a key role in the detoxification of superoxide anion and hydrogen peroxide thereby preventing ROS-induced damage (Fridovich 1995). The result from the study in Table 8 showed no hemolysis when compared with the control for the kidney lipid peroxidation and significant decrease when 5% of rat fed with diet compounded with seed oil were compared with the control group. The above observation shows that milk bush seed oil does not induce hemolysis at these percentages in the kidney. Finally, the result of catalase activity showed non-significant in both the liver and kidney when compared with the control group as shown in Table 9. Catalases are enzymes that catalyze the conversion of hydrogen peroxide to water and oxygen using either iron or manganese cofactor (Chelikani *et al*. 2004).

Table 1. Proximate analysis and physico-chemical properties of *Thevetia neriifolia* seed oil.

Parameter	Seed oil
Percentage of oil	58.03
Saponification value	70.125
Iodine value	4.145
Acid value	2.115

Table 2. Effect of milk bush (*Thevetia neriifolia*) seed oil on weight of organs of rats fed with varying percentages of the oil.

Treatment	Weight of liver	Weight of heart
Control	3.00±0.00	0.51±0.23
5% seed oil	2.96±0.90	0.40±0.25
10% seed oil	2.96±0.09	0.34±0.08
15% seed oil	2.60±0.55	0.36±0.14

Table 3. Effect of milk bush (*Thevetia neriifolia*) seed oil on body weight and feed intake of rats fed with varying percentage of the oil.

Treat-ment	Initial body weight	Final body weight	% Weight gain	Feed intake
Control	92.33± 10.32	140± 25.15	44.67± 51.6	112.74± 10.88
5% seed oil	86.67± 6.06	142± 25.15	55.33± 63.8	62.56± 18.82
10% seed oil	87.50± 5.24	103± 16.43*	15.50± 17.7	59.60± 19.86*
15% seed oil	85.83± 3.77	107± 10.95*	21.17± 24.7	56.11± 16.03*

There was a significant decrease in feed intake when 10% and 15% of rats fed with test diet were compared with the control group.

Table 4. Effect of milk bush (*Thevetia neriifolia*) seed oil on serum creatinine and protein determination of rats fed with varying percentage of the oil.

Treatment	Creatinine	Protein Determination
Control	2.98±1.63	5.54±0.08
5% seed oil	2.06±0.50	5.51±0.91
10% seed oil	0.24±0.90	7.08±1.80
15% seed oil	2.67±1.96	5.75±1.25

There was no significant change when rats fed with test diet were compared with the control group.

Table 5. Effect of milk bush (*Thevetia neriifolia*) seed oil on liver and heart cholesterol of rats fed with varying percentage of the oil.

Treatment	Treatment Liver Cholesterol mg/ml	Heart Cholesterol mg/ml
Control	154±38.40	24.80±2.29
5% seed oil	147.45±59.45	48.19±4.91
10% seed oil	91.97±32.34*	43.53±2.34
15% seed oil	111.77±36.37*	24.71±4.39

There was a significant decrease when 10% and 15% rats fed with the seed oil were compared with the control, while in heart lipid cholesterol the result showed a significant slight decrease when rats fed with 15% of the oil were compared with the control group.

Table 6. Effect of milk bush (*Thevetia neriifolia*) seed oil on liver and heart triglycerides of rats fed with varying percentage of the oil.

Treatment	Liver Triglycerides mg/ml	Heart Triglycerides mg/ml
Control	310.96±40.22	146.55±12.31
5% seed oil	261.56±48.17	118.09±26.06
10% seed oil	210.44±21.61*	107.80±45.40*
15% seed oil	247.99±103.21*	174.39±52.71

There was a significant decrease in liver triglycerides when rats fed with test diet compounded with the seed oil of 10% and 15% were compared with the control group.

Table 7. Effect of milk bush (*Thevetia neriifolia*) seed oil on kidney and liver SOD of rats fed with varying percentage of the oil.

Treatment	Kidney SOD units/mg protein	Liver SOD units/mg protein
Control	0.001±0.00	0.01±0.01
5% seed oil	0.008±0.00*	0.007±0.00
10% seed oil	0.008±0.01*	0.004±0.01
15% seed oil	0.006±0.00*	0.005±0.00

Table 8. Effect of milk bush (*Thevetia neriifolia*) seed oil on kidney and liver lipid peroxidation of rats fed with varying percentage of the oil.

Treatment	Kidney lipid peroxidation (MDA/mg protein)	Liver lipid peroxidation (MDA/mg protein)
Control	0.70±0.10	1.17±1.05
5% seed oil	0.87±0.46	0.36±0.20*
10% seed oil	0.73±0.67	0.85±0.69
15% seed oil	0.67±0.36	0.63±0.30

Table 9. Effect of milk bush (*Thevetia neriifolia*) seed oil on kidney and liver catalase activity of rats fed with varying percentage of the oil.

Treatment	Kidney catalase activity (µmol/H_2O_2) consumed/ min/mg protein	Liver catalase activity (µmol/H_2O_2) consumed/ min/mg protein
Control	125.97±0.94	121.72±14.34
5% seed oil	130.63±21.46	124.59±19.20
10% seed oil	99.43±22.80	113.29±24.27
15% seed oil	136.04±37.58	135.42±30.25

In Tables 2-9, the data were expressed as mean±S.D of six rats in each group and were analyzed using one-way student *t*-test. The use of the symbol '*' indicates that values for $p < 0.05$ were considered to be statistically significant when compared with the control.

Conclusion

The following conclusions can be drawn from the results of this study.

- Milk bush seed oil contains 58.03% oil on dry matter basis from the proximate analysis.

- Milk bush seed oil has acid value of 2.115 which falls in the range of edible oils.
- Milk bush seed oil does not increase the level of cholesterol which in excess is harmful to humans.
- There was mild increase in SOD activity in rats fed with the milk bush seed oil. This plays a key role in the detoxification of superoxide anion and hydrogen peroxide thereby preventing against ROS-induced damage.
- Therefore, milk bush seed oil meets the nutritional requirements and if detoxified could be recommended for consumption.

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