

Evaluation of safety of aqueous extract of *Tapinanthus sessillifolius* parasitic on *Psidium guava*

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Abstract

Plants products have been a source of food and medicines since antiquity. *Tapinanthus sessillifolius* commonly known as African mistletoe is used in lifestyle preventive medicine to enhance wellbeing and for treatment of various diseases. The heavy metals, macro elements were determined using Flame Atomic Absorption Spectrophotometer and some physio-chemicals were also evaluated for consistency of the extract. The acute and subchronic toxicity studies of aqueous fresh leaf extract of *Tapinanthus sessillifolius* was evaluated in albino mice and rats. This is to determine its safety profile by evaluating its effects on feed and water intake, body weight, relative organ weight and changes in some biochemical parameters after 21 days daily oral administration to rats. The results, estimated LD₅₀ to be greater than 2000 mg/kg/bw. The extract had no adverse effect on the efficiency of food and water consumption. Relative organ weight and the biochemical parameters tested were not significantly different $p < 0.05$ when compared to untreated animals. This was supported by histopathological studies of the organs where no adverse lesions were observed on tissues. However, there were lymphatic aggregates infiltration in one of the lungs rat treated with 800mg/kg. The toxic heavy metals, lead, cadmium and arsenic were not detected while moisture and ash were 7.02 % and 10.2% respectively falling within the permissible limit of WHO and RDA. Repeated oral administration of fresh leaf extract of *Tapinanthus sessillifolius* is relatively safe.

Keywords: *Tapinanthus sessillifolius*, *Psidium guajava*, safety, heavy metals, biochemicals.

Introduction

The use of medicinal plants as remedies for treatment of diseases, fitness, wellness and longevity are becoming more popular with the emergence of COVID -19 pandemic and other chronic ailments. Mistletoe with the common name(s) – bird lime, all heal, devil’s fuge, Iscador is an all heal plant [1,2,3]. Mistletoe is a general term for woody shoot parasites in several plant families, especially Loranthaceae and Viscaceae [4,5]. Most genera of African mistletoes belong to the family Loranthaceae (5,6). The seven genera of the Loranthaceae are *Helixanthera*, *Berhautia*, *Englerina*, *Globimetula*, *Agelanthus*, *Tapinanthus* and *Phragmanthera* with about five or more species are recognised in West Africa [7]. Worldwide, approximately 1500 species of mistletoe have been identified [7,8.]. Mistletoes are hemi-parasitic shrubs growing on dicotyledonous trees such as *Albizzia leb beck*, *Terminalia mantaly*, *Terminalia catappa*, *Khaya senegalensis*, *Citrus grandis*, *Cola acuminata* and *Theobroma cacao* [7]. Mistletoe attaches itself to the host by modified roots otherwise known as “haustorium” [5,6,7,]. Haustorium ensures the continuity of the macro elements sodium (Na), potassium (K), calcium (Ca) from the host to the parasite plant [8,9].

Infestation of mistletoes on smaller trees lead to poor growth and productivity and eventual death of host plants, especially during unfavorable weather conditions.

Ethnomedicinal uses of mistletoes had for a very long time been in the hands of very few herbal practitioners who claimed general use to sorcery and magical powers to treat mental conditions, sterility, and health problems associated with urino-genital system, rheumatism and pain. [4,8]. The leaves, stems, berries and flowers are the ones majorly used in herbal medicine for the treatment of headache, rheumatic pain, hypertension [10,11] ulcers [12] and cancers [13,14]. They are also acclaimed to possess hypoglycemic [15,16] lipid lowering [17] and antibacterial effects [18].

The chemical information readily available includes the structural polysaccharide and protein in *Phragmanthera capitata* leaf cell wall, the viscotoxins, carbohydrates and cytotoxic lectins. Compounds like alkaloids, flavonoids, cyanogenic glycoside (linmarin gallate) and walbruside were isolated [19]. Flavonoids namely, quercetin, catechin, qercitrin, rutin and avicularin have been isolated from the leaves of *Globimimetula braunii* [19,20]. The present study is to evaluate the acute and subchronic toxicity

effects of the aqueous fresh leaf extract of *Tapinanthus sessilifolius* growing on *Psidium guajava* host and also determine some quality standard makers of ash, moisture and toxic heavy metals.

Materials and methods

Plant material

The leaves of *T. sessilifolius* were harvested from the host, *Psidium guajava* in Jos, Plateau State of Nigeria. The plant material was identified and authenticated by Prof. Z. O. Gbile (Consultant Taxonomist, UNDP). Voucher specimen (No. FHI 105336) was deposited at Forestry Research Institute, Ibadan and at NIPRD, Idu, Abuja Nigeria.

Preparation of extract

Extraction of Plant *Tapinanthus sessilifolius*

The fresh plant material was cold extracted with distilled by disruption of the cell wall using blender. The extract was filtered through muslin cloth then filtered again through Whatman filter No. 42 and lyophilized using Lyovac GT2 (Germany). The yield was found to be (10.53 % w/w).

Physical Evaluation

Proximate analysis of extract

- A. Moisture - The determination of moisture content was based on the method of the Association of Official Analytical Chemist (AOAC) [20]. It is an indirect distillation method (evaporation of moisture). One gram (1.0 g) of the sample powder was weighed on aluminum foil on the automated moisture analysis pan (Model MB 200, OHAUS Florham PK.USA) and set at 105 °C for 3 hours after which percentage moisture content of the sample was obtained from the moisture balance
- B. Total Ash - The total ash was also determined by AOAC [20]. Porcelain crucible was washed and placed in the muffle furnace for 10 minutes to dry. After which it was removed and placed in a desiccator containing active desiccant to avoid moisture contact and allowed to cool. After cooling, it was weighed (M_1). 2 g of each of the sample were weighed in the crucibles (M_2) and was placed in the Muffle furnace at a 550 °C and allowed to stand 3 hours for complete combustion to ash to be achieved. The ash samples were removed placed in the desiccator for cooling and the weighed (M_3). The

percentage ash was calculated using the formula:

$$\% \text{ Ash (dry basis)} = \frac{M_{\text{ASH}}}{M_{\text{DRY}}} \times 100$$

C. Total proteins were estimated by the method of AOAC [20]

Phytochemical screening

The standard methods of Harbone (1998) and Agrawal and Paradhavi (2007) were used for preliminary phytochemical screening for phenols, tannins, saponins, flavonoids, alkaloids and terpenes [21,22].

Elemental analysis

Plant samples were prepared for elemental analysis as modified by AOAC [20]. The dried plant extract was ashed in oven electric muffle furnace maintained at 400 °C and 420 °C, for about 6 - 7 h to destroy all organic materials in the sample. The crucible containing pure ash was then taken out of the furnace and kept in a desiccator. Thereafter the ash was digested with triple mixture acids: sulfuric acids:sulphuric:perchloric acid (11:6:3) to obtain a clear solution. The solution was then made to 25 ml with double distilled water and read up with the flame technique of Hitachi Model 80-80 polarize Zeeman

Atomic Absorption Spectrophotometer (AAS) using air acetylene as fuel. The slit of 0.5 and appropriate lamps and their wavelengths were used. The mean value and the standard deviation of each element was determined by the AAS computer. Samples were analyzed in triplicate. Results obtained in part per million (PPM) were converted to milligram/100 gram as indicated below. Sodium and potassium were determined by flame fluorimeter.

In-vivo toxicity studies

Animals

Swiss albino mice (20 – 30 g) and Wistar rats (150 – 200 g) of either sex obtained and maintained at the Animal Facility Centre (AFC) of the National Institute for Pharmaceutical Research and Development, Abuja were used. All animals were housed under standard conditions of temperature (25 ± 2 °C) and light approximately (12/12 h light/dark cycle) and fed on standard diet and water *ad libitum*. These animals were approved for use by the ACE committee after reviewing the protocol for good laboratory practice and animal handling, which is in compliance with the National Institutes of Health *Guide for the Care and use for Laboratory animals* (Publication No. 85-23, revised 1985(23)).

Acute toxicity tests

The oral acute toxicity (LD₅₀) was determined in mice by a modified Lorke's [24] model. The test was carried out in five groups, each consisting of five (5) mice. Group I – IV were administered with varying doses of extract at 10, 100, 1000 and 2000 mg/kg i.p., while group V served as control and was administered normal saline at 10 ml/kg. Signs and symptoms of toxicity was observed over a period of 24 h. Death and sign of toxicity within this period were monitored was recorded.

Sub-Chronic toxicity Studies

Test Guidelines with slight modifications according to the Organization for Economic Cooperation and Development (OECD, 407) [25]. Healthy rats of either sex were randomly divided into four groups (n= 5). Animals received vehicle orally (water; control group) and aqueous extract in doses of 200, 400 and 800 mg/kg/day for 21 consecutive days. Body weight was recorded weekly as D1, D7, D14, D21. Food consumption and water intake were daily monitored. Daily feed (DFI) and Daily water intake (DWI) were determined [30] as:

$$DFI = \frac{\text{Weight of feed consumed by the group}}{\text{Total weight in the group}} \times 100$$

$$DWI = \frac{\text{Volume of water consumed by the group}}{\text{Total weight in the group}} \times 100$$

Animals were observed for signs of abnormalities during the whole treatment. Animals were fasted overnight after of food the last dose, but allowed free access to water. Blood samples were obtained by retro-orbital puncture, using capillary tubes for blood biochemical studies after which the animals were anesthetized by chloroform inhalation. The internal organs were isolated, weighed and fixed in formaldehyde for histopathological investigation. The relative organ weight (ROW) was calculated thus:

$$ROW = \frac{\text{Absolute weight of organ (g)}}{\text{Body weight of rat on day of sacrifice (g)}} \times 100$$

Statistical analysis: Data were expressed as Mean ± SEM Data for toxicity studies were analyzed by ANOVA followed by Dunnett's post hoc test for multiple comparisons and p<0.05 was considered significant.

Results

Phytochemical and Physicochemical analysis

The extract showed the presence of secondary metabolites such as the polyphenols, terpenoids saponin, glycosides. Alkaloids were not detected as shown in Table 1. Moisture, ash, carbohydrate,

protein and fats that were present are shown in Table 2. Table 3 represent the mineral elements and heavy metals that were not detected.

Table 1. Phytochemical compounds of aqueous fresh leaf extract of *Tapinanthus sessilifolius* on *P. guajava* host plant

| Phytochemicals | Polyphenols | Flavonoids | Terpenoids | Saponins | Alkaloids |
|----------------|-------------|------------|------------|----------|-----------|
| Remarks | ++ | ++ | ++ | ++ | - |

++ = Positive; - = Not detected

Table 2. Some physico-chemical parameters of aqueous fresh leaf extract of *Tapinanthus sessilifolius* on *P. guajava* host plant

| Parameter | Ash | Moisture | Lipids | Protein | Carbohydrate | Fibre |
|-------------------|-------|----------|--------|---------|--------------|-------|
| Concentration (%) | 10.02 | 7.02 | 16.32 | 5.56 | 54.8 | 7.3 |
| WHO | <8 | <10 | - | - | - | - |

Table 3: Mineral and heavy metal content of aqueous fresh leaf extract of *Tapinanthus sessillifolius*

| Elements | Na | K | Mg | Ca | Fe | Zn | Pb | Cd | As |
|----------------------|-------------|------------|--------------|--------------|-------------|--------------|--------------|----|----|
| (mg/100g dry weight) | 1.22 ± 0.01 | 32.1 ± 0.2 | 10.95 ± 0.01 | 50.55 ± 0.42 | 7.89 ± 0.02 | 13.42 ± 0.02 | 0.009 ± 0.01 | ND | ND |
| RDA mg | <2300 | 0.4-5.1 | 30-40 | 200-1300 | 0.2-27 | 2-15 | - | - | - |
| WHO | - | - | - | - | - | 50 | 10 | - | - |

RDA means recommended daily allowance (DRI, Food and Nutrition Board, 2000)

WHO = World Health Organization

ND = Not detected

Effect of leaf extract *T. sessillifolius* on acute oral administration in mice

The oral acute administration of the extract at varying doses of 10, 100, 1000, and 2000 mg/kg showed no any signs of toxicity. The behavioural pattern was similar in treated groups and control animals. No death was recorded in all animals. The acute lethal dose (LD₅₀) was therefore estimated to be greater than 2000 mg/kg body weight.

Effects on sub-chronic toxicity studies in rats

The daily oral administration of *T. sessillifolius* at doses of 200, 400 and 800 mg/kg bw. for 21 days did not show significant difference in body weight when treated animals were compared to normal control group even though there is increase in weight across all groups (Table 4). Also, there was no significant change in food and

water intake with administration of the extract. The relative organ weights of liver, kidney, lungs, spleen and pancreas treated rats were similar to control with no significant difference between the groups (Table 5). Treatment with the extract did not affect the levels of serum glucose or lipid profile as all the parameters testes were within normal range and not significantly different from untreated group (Table 6).

Histological analysis

Table 4. Effect of aqueous leaf extract of *Tapinanthus sessilifolius* (TS) on relative organ weight (g) in normal rats

Microscopic examination of the tissues of the internal organs did not show any change in the structure of the cells of liver, kidney spleen and pancreas of the treated animals that could be ascribed to the treatment of rats with the aqueous leaf extract of *T. sessilifolius*. However, lymphocytic aggregates were observed in one of the lung tissue in treated animals at doses of 800 mg/kg which were not present in control animals (Figure 1).

| Organ (g) | | | | | | |
|------------|-------------|-------------|-------------|-------------|--------------|-------------|
| TS (mg/kg) | Liver | Kidney | Lungs | Spleen | Pancreas | Heart |
| Control | 3.30 ± 0.10 | 0.96 ± 0.11 | 0.75 ± 0.14 | 0.37 ± 0.30 | 0.153 ± 0.20 | 0.35 ± 0.21 |
| 200 | 3.16 ± 0.20 | 0.91 ± 0.12 | 0.69 ± 0.13 | 0.34 ± 0.11 | 0.16 ± 0.09 | 0.32 ± 0.06 |
| 400 | 3.21 ± 0.11 | 0.89 ± 0.20 | 0.72 ± 0.21 | 0.33 ± 0.02 | 0.151 ± 0.06 | 0.33 ± 0.12 |
| 800 | 3.32 ± 0.11 | 0.94 ± 0.10 | 0.77 ± 0.12 | 0.32 ± 0.11 | 0.153 ± 0.2 | 0.33 ± 0.12 |

Data are expressed as Mean ± SEM (n = 5). All Values in test group are not significantly different from control (p<0.05) Two-way ANOVA Dunnett post hoc.

Table 5: Effect of Aqueous leaf extract of *Tapinanthus sessilifolius* (TS) on glucose and lipid profile in normal rats.

| TS (mg/kg) | Lipid profile (mg/dl) | | | | |
|----------------|--------------------------|-------------|-------------|-------------|-----------------|
| | Glucose | Cholesterol | HDL-cho | LDL-cho | Triacylglycerol |
| Control | 94.34 ± 2.1 | 97.00 ± 1.5 | 39.10 ± 1.3 | 39.70 ± 0.9 | 109.50 ± 1.3 |
| 200 | 95.82 ± 5.2 | 92.80 ± 5.2 | 38.90 ± 1.7 | 39.10 ± 1.5 | 102.50 ± 1.5 |
| 400 | 93.25 ± 2.2 | 89.80 ± 4.2 | 40.20 ± 0.8 | 39.10 ± 1.2 | 95.50 ± 1.3 |
| 800 | 98.33 ± 3.1 | 91.34 ± 1.7 | 39.1 ± 2.0 | 39.70 ± 1.5 | 107.03 ± 1.2 |

Data are expressed as Mean ±SEM (n = 5). All values in test group are not significantly different from untreated control (p<0.05). Two-way ANOVA Dunnett post hoc.

Table 6. Effect of aqueous leaf extract of *Tapinanthus sessilifolius* (TS) on some biochemical parameters in normal rats.

| TS (mg/kg) | ALT(UI) | AST(UI) | Ratio | Creatine (UI) | ALP | T.BIL |
|----------------|-------------|------------|-------|------------------|--------------|-------------|
| Control | 20.31± 0.70 | 39.6 ± 0.1 | 0.33 | 0.37 ± 0.30 | 0.153 ± 0.20 | 2.40 ± 0.31 |
| 200 | 19.5 ± 0.10 | 38.5± 0.12 | 0.45 | 0.34 ± 0.12 | 0.160 ± 0.09 | 2.32 ± 0.41 |
| 400 | 19.80 ±1.6 | 39.50± 0.2 | 0.51 | 0.33 ± 0.2 | 0.154 ± 0.11 | 3.12 ± 0.21 |
| 800 | 22.4 ± 0.5 | 40.20 ±0.1 | 0.55 | 0.32 ± 0.1 | 0.151 ± 0.1 | 3.20 ± 0.4 |

Data are expressed as Mean ± SEM (n = 5). All values in test group are not significantly different from untreated control (p<0.05). Two-way ANOVA Dunnett post hoc.

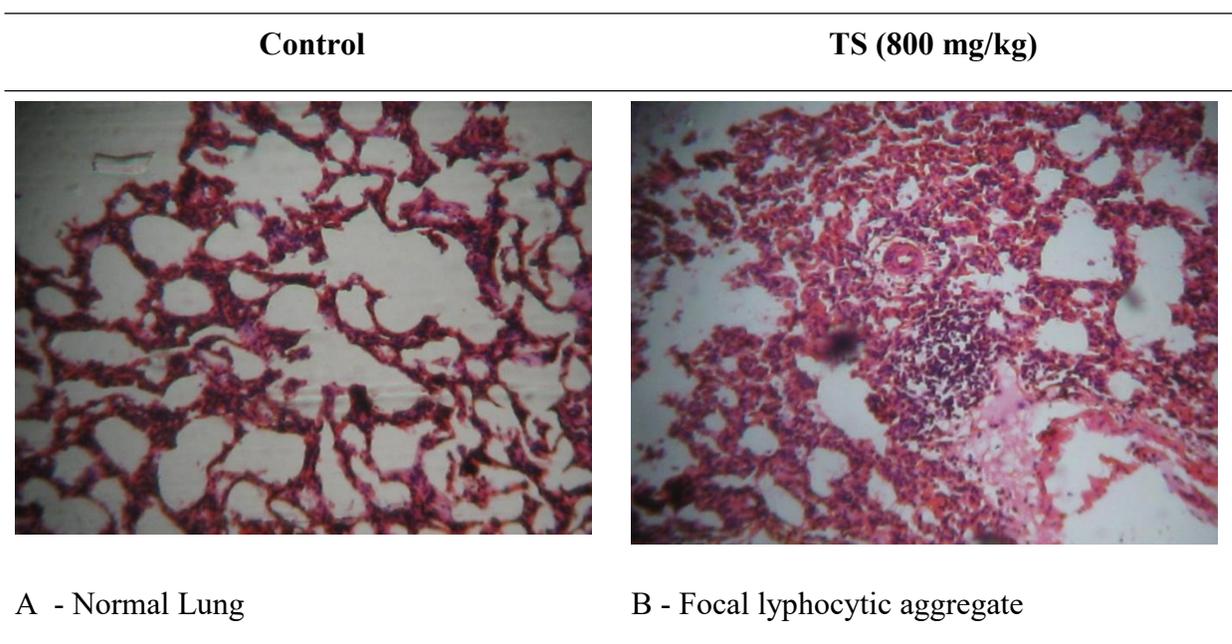


Figure 1 Representative slides of the histological analysis of the lung tissues of control (A) and treated (B) animals (Mag 20x10)

Discussion

Toxicological studies are generally done to determine the level of exposure and prevent the potential risk associated with the use of medicinal plants. The low level of moisture content that falls within the acceptable limit of 10 % will not promote microbial growth and subsequent biodegradation and instability of the bioactive components of the plants [27,28]. Fibre content of 7.3% is good for proper motility and faecal excretion.

Plants are a rich source of macro elements essential for human beings and they contribute to vital body function growth [29]. In this study, the inorganic essentials elements such as sodium (Na), potassium (K), calcium (Ca) and the trace elements that include iron (Fe), magnesium (Mg) and zinc (Zn) which are required for various metabolic processes in man were detected and the values falls within the WHO limits [43]. Adding to the integrity and safety of the extract is the absence of heavy metals

like Lead (Pb), Cadmium (Cd) and Arsenic (As). These metallic elements were not detected in this plant material as reported for other herbals [29,30].

According to reports by Rosidah 2009 [31] administration of 500 - 5000, and 5000 - 15000 mg/kg body weight is relatively safe and non-toxic respectively. Therefore, the lethal dose of greater than 2000 mg/kg of this study is relatively safe. The repeated administration of doses up to 800 mg/kg to normal rats did not produce observable toxic effects at the doses tested. This is consistent with other reports which showed that mistletoes extracts are relatively non-toxic [30,31,32]. However, in handling the plant material from raw material to extract, physicochemical reference like moisture and ash are critical so as to avoid deterioration, microbial and fungal growth that can be introduced as toxicants [33]. The extract is rich in polyphenols that are strong radical scavengers or antioxidants [34] but lack alkaloids which was reported by other studies on same host and difference species and this could be due to season and location of harvest [30;32] This is in agreement to the chemotaxonomic profiles [41].

The quality of many foods depends on the concentration and type of minerals and biomolecules they contain. The extracts

nutritional composition might have played a significant role against variety of degenerative diseases and processes [34]. Decrease in weight or excessive increase can be a pointer to toxicant in the system. However, the treatment with fresh extract the weight gain is not different from untreated even though not with food and water efficiency and relative organ weight. The astringency and pungency of the fresh extract would have caused the moderate consumption especially at higher dose of 800mg/kg body weight and the possibility of the mild lymphatic aggregates infiltration observed in the lungs histopathology of one in this treated group and this may be during oral administration liquid entered the lung. The liver and kidney play vital role in biotransformation and filtering blood from digestive tract to rest of the body [36] Therefore, any toxicants can cause inflammation and injury to the mitochondrial cells. It is known that elevated transaminase activities in conjunction with a rise in bilirubin level are considered as a marker index of hepatotoxicity linked to oxidant stress [35]. Increase in the aminotransferases in serum indicates cellular injury that leads to leaching of enzymes to serum before its manifestation with clinical histopathology

[36]. Reduced clearance due to increased proliferation of cells, increased rate of cell turn over and increased cell damage and increase in enzyme synthesis [37,38] This increase was not the case in this studies since the enzyme ratios were less than one because ratio of one indicates injuries and damage to liver [39]. It has been frequently reported that in the development of liver diseases there is an increased production of reactive oxygen species (ROS), often leading to greater hepatic lipid peroxidation. Lipotropic mediators and intracellular signals activate Kupffer cells, which initiate and perpetuate the inflammatory response and development of fibrosis and leaching of enzymes out to blood. [39,40,41]. The extract can be said to at no adverse effect level from the observed reports.

Conclusion

The administration of the aqueous fresh leave extract of mistletoe *T.sessilifolius* parasitic on *Psidium guava* is relatively safe within the limit of this studies. This validates its folkloric use of the plant for various ailments in humans and animals. Chronic studies and microbial load is required to ascertain gross safety.

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