# BIOLOGICAL AND PHYTOCHEMICAL INVESTIGATION OF EXTRACTS OF Tapinanthus bangwensis (ENGL. AND K. KRAUSE) DANSER [LORANTHACEAE] GROWN IN NIGERIA

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## ABSTRACT

*Tapinanthus bangwensis* is a parasitic woody shrub traditionally employed to cure various ailments in Nigeria including leprosy, rickets, rheumatism, cough, skin and respiratory diseases. The crude organic extracts of *T. bangwensis* were subjected to bioassays - cytotoxicity antibacterial and anti-diabetic. The cytotoxicity assay was carried out using brine shrimp lethality test (BSLT), the antibacterial activity was performed against five micro-organisms - *Escherichia coli, Pseudomonas aeruginosa*, *Klebsiellia rhinoscheromatis, Staphyloccocus aureus and Bacillus subtilis* using agar diffusion procedure while anti-diabetic potential was determined using alloxan-induced Swiss male albino rat with automated glucometer. The preliminary phytochemical investigation of the plant was done using standard procedure. $LC_{50}$ values for BSLT ranged from 12.38to 189.84 µg/mL with ethyl acetate extract of leaves having the lowest value12.38 µg/mL. The ethyl acetate and methanol extracts of both stem and leaves showed moderate to strong antibacterial activities. There was reduction of 71.8 % in the blood glucoses level of alloxan-induced rats on day 3 while glibenclimade gave 53.0 %. All the extracts exhibited significant reduction (P<0.05)in the blood glucose level of hyperglyceamic alloxan-induced rats. Phytochemical investigation of the extracts revealed the presence of tannins, steroids, anthraquinones and glycosides while alkaloids and flavonoids were absent.

**Keywords:** *Tapinanthus bangwens is*, Loranthaceae, African Mistletoe, Antibacterial, Cytotoxicity, Brine Shrimp Lethality Test.

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## INTRODUCTION

*Tapinanthus bangwensis* [Loranthaceae] is a parasitic, woody shrub, 1-2 m long, found in Western Central Africa. *T. bangwensis* is the commonest of the West Africa mistletoes and parasitizes a great number of trees such as cocoa, kolanut, rubber, pear, guava and orange. It is called 'Afomo' or 'Ose' in Yoruba; and African mistletoe in English. The leaf is used in Ghana to treat guinea worm infection. It is powdered up in Senegal for use externally in massages, plasters and baths for all enfeebling diseases (Burkil, 1985). It is also used for treatment of leprosy with *Gardenia triantha* (Rubiaceae) (Overfield *et al.*,1998).The decoction of the whole plant is used to cure respiratory diseases. It is also employed to treat sterility in cows and in medico-magical clinical treatment of impotence and to break spells. The leaves are used for treating skin-diseases, stiffness, rickets, fractured limbs, rheumatism, cough and chest pain. The powdered leaves are eaten to get rid of worms and to treat swelling (Gill and Onyibe, 1990). It has been reported that the leaves extract of this plant possess a good anti-inflammatory properties (Patrick-Iwuanyanwu, *et al.,*2010). The leaves contain mineral elements, fiber, protein and carbohydrate (Bassey*, et al.,* 2012).Diabetes mellitus has been a disease that affects a lot of populace which may be due to excess sugar in system as a result of no enough insulin in the body especially Type 2 diabetes (Osadebe *et al.,* 2010). These ailment open doors to different diseases that ranges from heart failure, stroke, paralysis and hypertension (Obatomi and Aina*,* 1996; Osadebe *et*





*al*., 2010).The prevalence of *Diabetes mellitus* is on increase throughout the world. This is due to different factors which include obesity, ageing and stress. *Diabetes mellitus* has been reported to be the sixth ailment which leads to mortality (Qamar *et al.,* 2012). Due to crisis pose on human due to *Diabetes mellitus*, the research is geared towards evaluating the plant for its anti-diabetic, anti-bacteria and toxicity level.

## Materials and Methods

*Plant materials*

Fresh leaves of *Tapinanthus bangwensis* were collected on kola tree (*Cola acuminata*) growing naturally on the main campus of Olabisi Onabanjo University, Ago-Iwoye, Ogun State, Nigeria. This was identified at the Department of Botany, University of Ibadan and authenticated at the Forestry Research Institute of Nigeria (FRIN), Ibadan. The voucher specimen was deposited at the Department of Botany, University of Ibadan as UIH 22399.

### *Plant Extraction*

The air-dried leaves and stem of *T. bangwensis* were pulverised and extracted with hexane (1.5 L), ethylacetate (1.5 L) and methanol (1.5 L) successively by cold extraction. This was carried out by soaking the powdered leaves in the solvent for 72 hrs and the solvent was filtered. The extracts obtained were concentrated using rotary evaporator at 40$^o$Candsubsequently keptinside desiccators prior to use.

### *Phytochemical Studies*

The preliminary phytochemical analysis of the plant extracts for various secondary metabolites such as saponins, alkaloids, tannins, anthraquinones, reducing sugars, steroids, glycosides and flavonoids were done using standard procedures of analysis (Trease and Evans 1989, Harborne 1998, Edeoga *et al.,* 2005). Each of the tests was qualitatively expressed as negative (-) or positive (+).

### *Brine shrimp lethality test* (BSLT)

The assay was performed according to Meyer's method (Meyer *et al.,* 1982). Briefly, brine shrimp eggs (*Artemia salina*) were placed on one side of a small vessel which was filled with filtered sea-water and fully aerated for 48 hours at room temperature. The nauplii (hatched shrimp) were attracted to the other side of the vessel with a light source. The samples were prepared in concentrations of 10,000, 1000and 100µg/mL in dimethyl sulphoxide (DMSO). About 2mL of the samples at each concentration was put into test tubes and 5ml of sea-water was added. To these solutions, 10 nauplii were added per vial and made up to 10ml with sea-water. Tests for each concentration were carried out in triplicates. The vials were maintained under light. Survivors were counted after 24 hrs and the percentage mortality in each vial and control was determined using the equation:

$$\%\ Mortality = \left(\frac{no.of\ dead\ nauplii)}{(initial\ no.of\ live\ nauplii}\right) \times 100$$

The data results obtained were analysed statistically using Probit analysis by Finney (1971), to determine the $LC_{50}$ values.

### *Antibacterial Bioassay*

Five standard strains of human pathogenic microorganisms, which consisted of three gram-negative bacteria (*Escherichia coli* ATCC 25122, *Pseudomonas aeruginosa* ATCC 27853and *Klebsiellia rhinoscheromatis* UCH 2049) and two gram-positive bacteria (*Staphyloccocus aureus* ATCC 13709 and *Bacillus subtilis* PHM 1502) were used for the study.

Nutrient agar and nutrient broth, from Oxford laboratories UK were used in the assay. Gentamycin sulphate (1mg/mL) was used in the test as the standard reference drug. All extracts were reconstituted in the hexane, ethyl acetate and methanol. The agar cup broth procedure was employed. An overnight broth culture of 1.2 x $10^7$ CFU of each bacterium was used to seed sterile molten agar medium maintained at 45$^o$C. When seeded plates were set, six wells (8 mm in diameter) were bored in each plate with an aseptic cork borer. Two concentrations (20 and 100 mg/mL) of all the extracts were prepared and 80 µL of the two different concentrations were dispensed into each of the wells with the aid of pasteurised pipette. This was carried out for the hexane, ethyl acetate and methanol which served as blank control and gentamycin – the positive control. Zones of inhibition were determined measured in millimeter (mm) after incubating plates at 37°C for 24 hours.

***Anti-diabetic assay***

**Experimental Animals**

Single sex (male) Swiss albino rats were used. They were purchased from the Department of Pharmacology, University of Ibadan, Oyo state. Their average weight ranges between 120-150g. They were acclimatized for three weeks and fed with standard pelletised feed (Ladokun feed) and allowed water *ad libitum.*

**Animal grouping**

**Group A:** untreated control rats

**Group B:** alloxan-induced diabetic rats that were not treated with the extracts nor glibenclamide (negative control)

**Group C:** alloxan-induced diabetic rats treated with 50 mg/mL extracts

**Group D:** alloxan-induced diabetic rats treated with 100 mg/mL extracts

**Group E:** alloxan-induced diabetic rats treated with 200 mg/mL extracts

**Group F:** alloxan-induced diabetic rats treated with glibenclimade 2.5 mg/kg (positive control)

The rats were kept into different cages with six rats of about the same size in each group. After the grouping, the fasting blood glucose levels of the rats were then measured with glucometer by letting blood from the tail (Osinubi *et al.,* 2008; Mohan *et al.,* 2010). Alloxan monohydrate was injected into the rats intraperitoneally for the induction of diabetes in the rats. The induction was carried out for seventy two hours and after which another blood glucose level was taking which revealed that the blood glucose level of the rats were already increased. The extract was prepared in three different concentrations which are 200, 100 and 50 mg/mL were administered orally to the rats for 3 days. The rats were given 1 mL of the prepared extract concentration twice in the first day and once in the other days with their blood glucose level measured daily. The anti-diabetic activity was determined in triplicates to ascertain the level of blood glucose released. Glibenclamide served as reference standard drug (positive control) while DMSO was used as negative control (normoglycaemic). Blood sample were collected from the rats through retro orbital plexus puncture method. The blood glucose levels were determined and estimated using AccuChek™ and glucose strips in AccuChek™ test metre. The results obtained were statically analysed using SEM and ANOVA at confidence limit of P<0.05.

**Results**

**Phytochemical Screening Result**

The phytochemical screening result is presented in Table 1 below. There was the presence of anthraquinones in TBLE, TBSE and TBLM. Glycosides were present in TBLH, TBSH, TBLM and TBSM. Saponins are present in TBLH, TBSH, TBLM and TBSM while tannins are found in TBLE, TBSE, TBLM and TBSM. Steroids were only found in TBLM and TBSM. There was absence of alkaloids and flavonoids in all the extracts.

**Table 1: Phytochemical Screening of Extracts of *Tapinanthus bangwensis* Leaves and Stem**

| Plant Extract | Alkaloids | Saponins | Tannins | Anthraquinones | Glycosides | Steroids | Flavonoids |
|---|---|---|---|---|---|---|---|
| TBLH | - | + | - | - | + | - | - |
| TBSH | - | + | - | - | + | - | - |
| TBLE | - | - | + | + | - | - | - |
| TBSE | - | - | + | + | - | - | - |
| TBLM | - | + | + | + | + | + | - |
| TBSM | - | + | + | - | + | + | - |

**+ = Metabolite detected; - = Metabolite not detected**

TBLH – Hexane extract of *Tapinanthus bangwensis* leaves

TBSH – Hexane extract of *Tapinanthus bangwensis* stem

TBLE – Ethylacetate extract of *Tapinanthus bangwensis* leaves

TBSE – Ethylacetate extract of *Tapinanthus bangwensis* stem

TBLM – Methanol extract of *Tapinanthus bangwensis* leaves

TBSM – Methanol extract of *Tapinanthus bangwensis* stem.





**Brine Shrimps lethality test Result**

The brine shrimps lethality result which show the cytotoxicity activity of the plant's extracts is shown in Table 2. The extract of TBLE was found to possess high cytotoxicity followed by TBSE, TBLM and TBSM. The cytotoxicity activity was found to be concentration dependent.

**Table 2: Cytotoxic activity of *Tapinanthus bangwensis* extracts against brine shrimps.**

| Plant extracts | % Mortality at Different Concentration* | | | $LC_{50}$ |
|---|---|---|---|---|
| | 100 μg/mL | 1,000 μg/mL | 10,000 μg/mL | μg/mL |
| TBLE | 50.0 | 63.3 | 76.7 | 12.38 |
| TBSE | 56.7 | 66.7 | 100 | 97.32 |
| TBLM | 43.3 | 66.7 | 83.3 | 188.57 |
| TBSM | 46.7 | 60.0 | 83.3 | 189.34 |

* Mean of three determinations.

**Antibacterial result**

Table 3 the antibacterial activities of *Tapinanthus bangwensis* extracts. TBLH, TBLM and TBSH did not show any inhibition against all the microorganisms. TBLE only inhibit the growth of *Bacillus subtilis* while TBSE INHIBIT *Staphylococcus aureus* and *Bacillus subtilis.* TBSM was found to inhibit the growth of all the microorganisms.

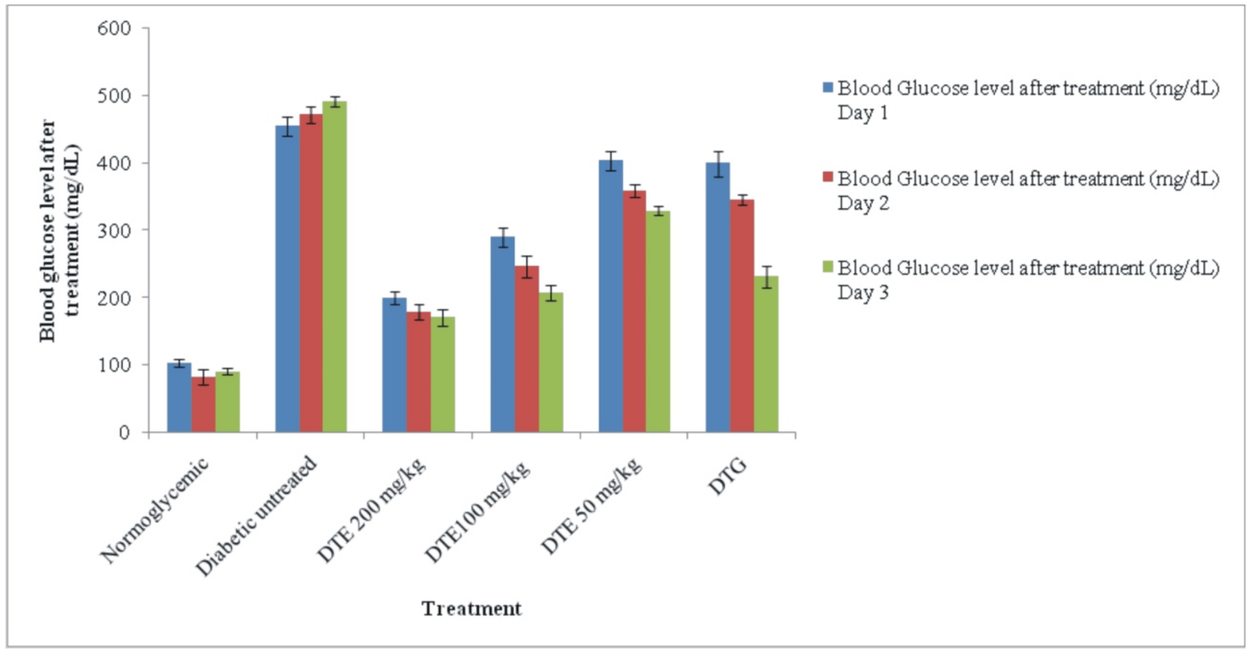
**Table 3: Antibacterial activities of extracts of *Tapinanthus bangwensis* leaves and stems**

| | Microorganisms/Diameter of Zones of inhibition (mm) | | | | | |
|---|---|---|---|---|---|---|
| **Extracts** | **Extract conc. (mg/ml) Ref/Control** | **S. au** | **E. co** | **B. su** | **P. ae** | **K. rh** |
| TBLH | 20 | 0 | 0 | 0 | 0 | 0 |
| | 100 | 0 | 0 | 0 | 0 | 0 |
| TBLE | 20 | 0 | 0 | 0 | 0 | 0 |
| | 100 | 0 | 0 | 12 | 0 | 0 |
| TBLM | 20 | 0 | 0 | 0 | 0 | 0 |
| | 100 | 0 | 0 | 0 | 0 | 0 |
| TBSH | 20 | 0 | 0 | 0 | 0 | 0 |
| | 100 | 0 | 0 | 0 | 0 | 0 |
| TBSE | 20 | 12 | 0 | 10 | 0 | 0 |
| | 100 | 16 | 0 | 15 | 0 | 0 |
| TBSM | 20 | 11 | 0 | 10 | 12 | 10 |
| | 100 | 13 | 12 | 15 | 10 | 14 |
| | Blank control | 0 | 0 | 0 | 0 | 0 |
| | Gentamycin | 16 | 12 | 20 | 12 | 18 |

Key: S. au = *Staphylococcus aureus* ATCC 13709; E. co. = *Escherichia coli* ATCC 25122
B. su = *Bacillus subtilis* PHM 1502 P. ae = *Pseudomonas aeruginosa* ATCC 27853
K. rh = *Klebsiellia rhinoscheromatis* UCH 2049

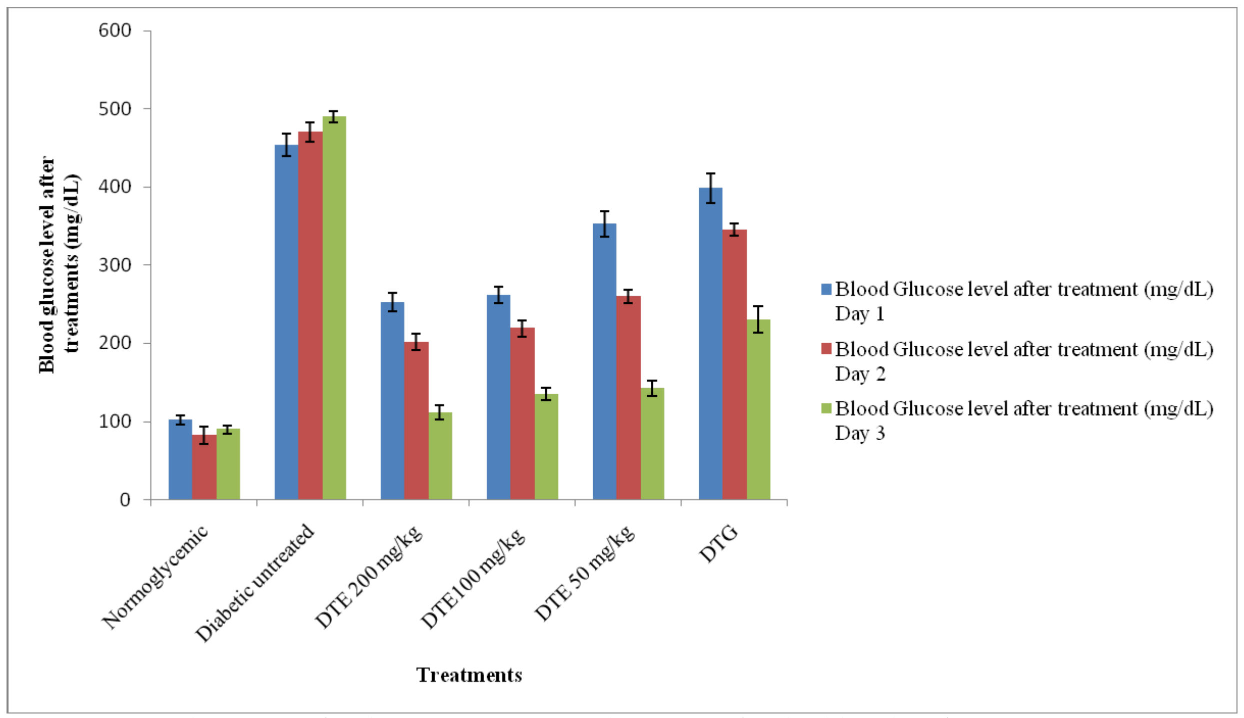
**Antidiabetic result**

The antidiabetic results are represented by Figure 1.0 and 2.0 below. The antidiabetic activities of the plant were found to be dose dependent. There was drastic reduction in the glucose level of the animal treated with 200 mg/kg extract compared to the glucose level of the animal treated with glibenclamide. This trend was observed with the two extracts (TBLE and TBSM).




*Key: DTE = Diabetic Treated with Extract; DTG = Diabetic Treated with Glibenclamide*

***Figure 1.0:Antihyperglyceamic effect of TBLE extract***




*Key: DTE = Diabetic Treated with Extract; DTG = Diabetic Treated with Glibenclamide*

***Figure 2.0: Anti-hyperglyceamic effect of TBLM extract***



**Discussion and Conclusion**

The results of phytochemical screening of the extracts of *Tapinanthus bangwensis* leaves and stem revealed that Saponins, tannins, anthraquinones and glycosides are present in the extracts while No alkaloids, reducing sugar and flavonoids were detected in all the extracts. The methanol extract of *T. bangwensis* leaves and stem shows the presence of steroids. Glycosides could be used as cardiac stimulant and tannins may be hydrolysed to obtain propyl gallate, which is a strong antioxidant (Shazid *et al.*, 2009; Ann *et al.*, 1998). Tannins are also known to possess antimicrobial properties. At low concentration, tannins can inhibit the growth of microorganisms and act as an antifungal agent at higher concentration by coagulating the protoplasm of the microorganism (Adekunle and Ikumapayi, 2006). Anthraquinones possess antiparasitic, bacteriostatic, antidepressant and antimicrobial properties (Cowan, 1999). Brine shrimp lethality test has been a vast method used to screen both the crude extracts of plants and isolated pure compound for preliminary toxicity before adopting cancer cell line method. The test indicates cytotoxicity as well as a wide range of pharmacological activities such as antimicrobial, pesticidal and antitumor activities, which has led to the isolation of some pesticidal and anticancer agents (Anderson *et al.,* 1988). It was shown from the results that all the extracts were lethal against the brine shrimp. The percentage mortality being concentration dependent. The variation observed in the results (Table 2) may be due to differences in the amount and kind of cytotoxic constituents of the crude extracts such as tannins, flavonoids or steroids. Moreover, the significant lethality of the crude extracts ($LC_{50}$ value less than 1000ppm or µg/mL Meyer, 1982; Khaled, 2006) is indicative of the presence of potent cytotoxic compounds, hence all the extracts are good cytotoxic agents. Therefore the positive response obtained in this assay suggests that the extracts could be promising antitumor, antibacterial or pesticidal compounds (Akpemi, 2012). Also, since the extracts have been shown to exhibit cytotoxic effect, there is a need for caution by traditional care givers regarding prescription of these plants for therapeutic purposes. However, it will be very imperative to also carry out cancer cell line assay to really know the level of this toxicity in humans. The result confirmed what has been reported in literature that the berries of African mistletoe are toxic (Adodo, 2004). The hexane extracts showed no activity on all the organisms. Ethyl acetate extracts of leaves showed moderate activity on *Bacillus subtilis*. The methanol extract of stem (TBSM) was active on the entiremicroorganismusedat100mg/mLwhile ethyl acetate extract of stem only inhibited *Staphylococcus aureus* and *Bacillus substilis*. The inhibition of methanol extract of stem was similar to that of *Phragmantera incana* as reported by Atewolara-Odule and Aiyelaagbe, 2013. It is interesting to note that the activity of the TBSE and TBSM extracts at 100 mg/mL is comparable to that of the control drug, gentamycin. The results suggest that this plant may be used to treat diseases associated with the tested microorganisms. The result corroborate Ekhaise *et al.*, 2010, however, this is reporting the plant inhibiting Bacillus substilis. The antidiabetic properties of *T. bangwensis* are represented in figure 1.0 and 2.0. The diabetic rats had much higher blood glucose level than that of normal control. The result showed an increase in the blood glucose level of rats following the administration of Alloxan. All the extracts displayed a good reduction in the blood glucose of rats except Day 1 at concentration of 50 mg/kg where no reduction in the blood glucose was observed. The highest reduction in the blood glucose level of rats was observed with dosage of 200 mg/kg. This implies that the reduction in the blood glucose of the rats was concentration dependent. The result also showed that, at 200 mg/kg of the extracts, there was drastic reduction in the blood glucose of the rats treated with the standard drug glibenclamide. TBLM showed more activity than TBLE. There was reduction of 71.8 % in the blood glucoses level of alloxan-induced rats on day 3 while glibenclamide gave 53.0 %. From the result, it was observed that all the plants' extracts exhibited significant reduction at P<0.05 in the blood glucose level of hyperglyceamic alloxan-induced rats. Of great importance is that the extracts of the plant displayed a better reduction than glibenclamide. The findings suggest that the plants extracts could be a source of insulin production and glucose utilisation just like glibenclamide, to cause reduction in the blood glucoses. The result obtained is similar to the anti-diabetic effect of *Tapinanthus butungil* in alloxan-induced Sprague-Dawley rats (Osinubi *et al.,* 2008) and anti-diabetic effect of *Loranthus micranthus* (Osadebe *et al.,* 2010). This finding is in line with the folkloric usage of the plant as an anti-diabetic and its cytotoxicity justified its use as an anti-cancer (Grossarth-Maticek *et al.,* 2007).

The plant extracts demonstrated cytotoxic activity is a signal of showing wide range of healing activities which may include anticancer, antiviral and pesticidal properties. The extracts further displayed antibacterial activities which could be due to the presence of the secondary metabolites in the extracts such as tannins. The antihyperglyceamic displayed by the extracts establish the traditional use of the *T. bangwensis* as an anti-diabetic plant.

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