# Acute Toxicity and Anxiolytic Activity Screening of Hydroalcoholic Leaf Extracts of *Bryophyllum pinnatum*, *Terminalia catappa* and *Tapinanthus dodoneifolius* Growing on *Terminalia catappa* Tree in Mice




## ABSTRACT

**Aims:** Hydroalcoholic *T. dodoneifolius*, *B.pinnatum* and *T. catappa* leaf extracts were investigated for their acute toxicity and anxiolytic activities.
**Study design:** Acute toxicity (LD50) was determined using the limit dose acute and Anxiolytic activities were assessed by open-field field behavioural testing in mice.
**Place and duration of study**: The study took place in the Neurobehavioural room of Department of Pharmacology & Therapeutics, Faculty of Pharmacy, Ahmadu Bello University, Zaria in late December 2022..
**Methodology:** Groups of mice (n=6; equal sexes) were each exposed to the open-field paradigm (OFT) following 1 hour of single oral administration of distilled water, extracts (125, 250, and 500 mg/kg) and diazepam (0. 5 mg/kg) using the behavioural indices of mean centre zone time (M%CZT), centre zone re-entry (MCZR) and defeaction/urination frequency (MD/UF).
**Results:** Acute toxicity testing shows that the three extracts are safe with LD50 of 5g/kg. Behavioural results indicate, compared to the distilled water treatment (M%CZT, 6.39±1.53; MCZR, 4.67±1.15, & MU/UF, 4.83±0.75), single acute *T. dodoneifolius* (M%CZT, 7.50±1.73, 13.72±2.43, & 20.94±3.91*; MCZR, 7.33±0.88, 9.50±0.76, & 11.50±1.6*; MD/UF, 4.17±0.48, 3.17±0.54, & 1.83±0.60*), and *B.pinnatum* (M%CZT, 9.72±1.91, 9.78±1.32, & 19.67±2.01*; MCZR, 6.00±1.15, 10.50±0.76, & 12.50±1.72*; MD/UF, 3.17±0.79, 2.33±0.42, & 1.33±0.49*) extract treatments demonstrated consistent dose-dependency in their anxiolytic activity across the three parameters with *T. catappa* extract (M%CZT, 6.95±1.27, 12.17±2.01, & 16.84±1.49*; MCZR, 8.17±1.60, 10.17±1.67, & 10.83±1.96; MD/UF, 2.83±0.60, 2.00±0.73, & 3.67±0.42) exhibiting dose-pendent anxiolytic effect only on the M%CZT. However, at the highest dose level of 500 mg/kg, all plant extract treatments caused anxiolytic effects that are comparable with those of 0.5 mg/kg diazepam treatments (M%CZT, 22.61±1.31*; MCZR, 12.17±1.66*; & MD/UF, 2.62±0.21*).
**Conclusion:** These findings justify the traditional use of these medicinal plant extracts in the remediation of nervous and related disorders.

**Keywords:** Acute, Anxiolytic, Open-field test, Centre zone time, Centre zone re-

entry, Mice, Defeacation, Urination

## 1. INTRODUCTION

Anxiety disorders are chronic psychobehavioural states that are characterized by inappropriate, inexplicable and/or excessive fearfulness, tension, and irritability – all of which may be disabling to the victims [1; 2; 3]. The various forms of these mood disorders – generalized anxiety disorder (GAD), obsessive-compulsive disorder (OCD), panic anxiety disorder (PD), social anxiety disorder (SAD) and post-traumatic stress disorder (PTSD) – collectively constitute a high disease prevalence and socio-economic burden [4; 5; 6]. This scenario is heightened by the paucity, toxicity and effectiveness concerns of the currently available anti-anxiety therapeutic agents [7; 8]. Therefore, to both meet the increasing demand of for these disorders and mitigate the afore-mentioned anti-anxiety therapeutic and clinical liabilities, there is a need to discover new agents - preferably with novel anxiolytic mechanism(s) to complement and/or substitute the traditional benzodiazepine– and serotonin- related drugs [9; 10; 11].

The plant kingdom has been a limitless source of new pharmacophores for diverse disease spectra [12; 13]. Anti-anxiety agents are not an exception to this – with anxiety-relieving activity and the presence of phytochemicals reported for some medicinal plants including *Hydrocotyle umbellata* L., *Ginkgo biloba*, *Piper methysticum*, *Camellia sinensis*, Leonurus cardiac, *Valeriana officinalis*, *Withania somnifera*, *Nymphaea alba*, *Passiflora incarnata*, *Commelina benghalensis*, *Turnera aphrodisiaca* and *Crataegus oxyacantha* in both animal and human studies [14; 15; 16]. There is a need, therefore, to search the plant kingdom further to discover new anxiolytic agents. Thus, based on the afore-mentioned ethno-medicinal grounds, this study set out to assess the anti-anxiety potential activity of leaf extracts of *B. pinnatum*, *T. catappa* and *T, dodneifolius* growing on *T.* tree.

*B. pinnatum* (Lam.) Pers. (syns: *Bryophyllum calcicola, Kelanchoe pinnata*, *Bryophyllum calcinum*, *Cotyledon calcina*, *Bryophyllum germinans,* etc.) commonly known as miracle leaf, air plant, life plant, Goethe plant and cathedral bells, and of the family Crassulaceae, is a plant that is now naturalized to the tropical and subtropical regions of the world [17]. This perennial plant, which is increasingly becoming an ornamental item in Nigerian households, has a fleshy cylindrical stem that can grow up to 1 meter tall with succulent, fleshy, elliptical-shaped, curved and margin-serrated deep green leaves [18].

Plate 1: Bryophyllum pinnatum

Reports from Nigeria and other parts of the world show *B. pinnatum* and its congeners are used ethno-medicinally for the treatment of several illnesses including gastric ulcer, hypertension, smallpox, cancers, migraines, diabetes mellitus, psychosis, colds, dysmenorrhea, hepatitis, diarrhea and dysentery, ear infection, kidney stones, conjunctivitis, retained placenta, chicken pox, rheumatoid arthritis, pulmonary infection, heart failure and palpitation [19; 20; 21; 22; 23; 24].

Reported pharmacological activities of extracts of *B. pinnatum* and its congeners include anti-hypertensive, hypoglycemic, nephroprotective,

hepatoprotective, antibacterial, immunosuppressive, analgesic, anti-inflammatory, insecticidal and anti-carcinogenic effects [24; 25; 26; 27; 28; 29; 30; 31; 32]. These biological activities are viewed to be due to the presence of several important phytochemicals (4-hydroxy-3- methoxy-cinnamic acid, bufadienolide, protocatechuic acid, epigallocatechin-3-o-syringate, quercetin, 4',5-dihydroxy-3',8-dimethoxy flavone-7O-β-D-glucopyranoside, β-amyrin, 22-dihydrobrassicasterol, bryophyllin A, oxalic acid, malic acid), vitamins (riboflavin, thiamine, pyridoxine, ascorbic acid, niacin), minerals (calcium, magnesium, zinc, manganese, copper, potassium, iron) and amino acids (glutamic acid, glycine, cysteine, tyrosine, casein hydrolysate, phenylalanine, protein hydrolysate, methionine) [33; 34; 35; 36] in these extracts.

Previous studies have shown extracts of *B. pinnatum* and its congeners to exert dose-dependent anticonvulsant activity, antidiabetic, anxiolytic, hypnotic and muscle relaxant effects in rodents [37; 38], and antidiabetic and anxiolytic effects in young Zebra fish [40]. However, scientific reports on the anxiolytic activity of *k. pinnata* are scanty and this study, therefore, aims to investigate its acute anxiolytic effect in mice in the open field test.

*Terminalia catappa* L. (syns: Phytolacca javanica (Osbeck); *Terminalia mauritiana* (Blanco); *Terminalia moluccana* (Lamk.); and *Terminalia procera* (Roxb)) is a medium-large sized Tropical tree plant, commonly known as Tropical almond, belonging to the combretum family. The plant at full maturity has a thick stem with dark brown/grey fissured bark; stem branches bearing spirally arranged simple, broadly obovate, round and blunt tipped leaves; stalkless, ovoid to ovate thin-skinned fleshy fruits each containing a seed enclosed within a hard shell; and smallish five-lobed, axillary-spiked mildly unpleasant smelling white or cream coloured male-dominated flowers [41]. In Nigeria, the fruit of this plant is consumed orally as snacks. Ethno-medicinally, different parts of *Terminalia catappa* have been widely reported to be helpful in the treatment of leprosy, headache, dermatitis, abdominal colic, fever, gastropeptic dyspepsia, hepatitis, urinary tract infection, diarrhea, heart failure and poor sexual drive [42; 43; 44; 45; 46]. In addition, extracts from different parts of this plant have been reported to exhibit antidiabetic, anti-lipidemic, antineoplastic, hepatoprotective, aphrodisiac, anti-inflammatory, antibacterial, antioxidant, analgesic and anti-ageing effects [44; 45; 47; 48; 49; 50; 51; 52]. But scientific literature has scanty reports on the anxiolytic activity of extracts from *T. catappa*. Thus, this study aims to screen the aqueous methanol leaf extract of this plant for its acute anxiolytic activity employing the mouse open field test.

The third medicinal plant whose leaf extract that will be investigated in this study is *Tapinanthus dodoneifolius* (DC.) DENSER (syns: *Agelanthus dodonaeifolius*; *Dentimetula dodonaeifolius*; *Loranthus chevalieri*; *Loranthus dodoneifolius*; *Loranthus knoblercheri*; *Loranthus uelensis* and *Scurrula dodonaeifolia*) [54].

Plate 2: Terminalia catappa

*Terminalia catappa* (Almond) tree hosting *Tapinanthus dodoneifolius* epiphytes This plant is a green leafy shrub found in most parts of Nigeria and the Tropics growing as a parasitic plant on host trees such as *Theobroma cacao*, *Tamarindus indica*, *Acacia nilotica*, *Vitelleria paradoxa* (Shea butter), Kolanut, *Parkia biglobosa*, *Terminalia catappa*, *Citrus sinensis* and *Azardirachta indica*. It is known as Viscum album in Europe, mistletoe in English, afomo onisana in Yoruba, and Kauchii in Hausa [55; 56]. Its extracts have been reported for efficacy in diverse diseases including hypertension, menstrual pain, diabetes mellitus, cancers, abdominal colic, gonorrhea, malaria, diarrhea, dysentery and peptic ulcer [57; 58; 59;

60; 61]. In addition, several important pharmacological activities which have been associated with extracts obtained from *Tapinanthus dodonaeifolius* and its synonyms include antimicrobial, anti-ulcerogenic, antiplasmodial, antihypertensive, anticancerous, anticonvulsant and hepatoprotective [55; 57; 58; 59; 60; 61; 62; 63]. These biological activities may be due to the presence of numerous important phytochemical compounds such as anthraquinones, saponins, tannins, alkaloids; phlobatannins, carbohydrates, flavonoids, anthracene, cardiac glycosides, steroid and triterpenes, and a dihydropyranone Dododeine [58; 59; 60; 61; 67]. Previous studies indicate a bark extract of *Tapinanthus dodonaeifolius* to have exhibited anxiolytic, antidepressant and spatial memory-enhancing effects and a hydromethanol leaf extract and its fractions of *Tapinanthus globiferus* epiphytic on *Azardirachta indica* tree to have demonstrated an anxiolytic activity [64; 65; 66]. But scientific reports on the anxiolytic activity of the leaf extract of *Tapinanthus dodoneifolius* are scarce. This study is to investigate the acute anxiolytic activity of *Tapinanthus dodoneifolius* in mice using the mouse open field test.

## 2. MATERIAL AND METHODS

### *2.1 Drugs, Open-field apparatuses and Plant materials*

Diazepam tablets (Roche, 5 mg) were obtained from a pharmaceutical outfit near the Ahmadu Bello University campus, Zaria. Units of 40 x 40 x 40 cm transparent plexiglass open-field apparatus and distilled water were graciously supplied by the Department of Pharmacology laboratory, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria.

*B. pinnatum* fresh leaves were harvested in Then, the Gwagwalada area of the Federal Capital Territory, Abuja. Freshly fallen leaves of *T. catappa* and fresh leaves of *T. dodoneifolius* growing on *T. catappa* were harvested on the main campus of Ahmadu Bello University, Zaria, Nigeria, all in December 2022. Following collection, all leaves were each separately briskly washed to remove dirt and contaminants. Then, *T. dodoneifolius* and *T. catappa* leaves were separately air-dried until complete dryness while, due to the difficulty of getting it to dryness through air-drying on account of their succulent nature, *B. pinnatum* leaves were first pulverized to fine texture using an electrical blender, following which it was thinly spread on a clean flat inert white surface and air-dried by the aid of an electrical fan until the constant dry weight was achieved. All dry leaves were ground to fine powders and kept in plastic containers at room temperature till use. Twenty grammes (21 g) of dry *B. pinnatum* leaves, and 40 g each of dry *T. dodoneifolius* and *T. catappa* leaves were each soaked in 0.5 L of equal volumes of distilled water and ethanol for 24 hours, following which the macerates were separately Whatman paper-filtered. The resulting filtrates were separately air dried aided by electric fanning.

### *2.2 Animals*

Healthy adult white albino Swiss mice of both sexes were sourced from the animal house of the Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Kaduna State, North-west Nigeria. They were kept in clean plastic battery cages for at least 7 days under room temperature under 12-hour light/dark cycle with

access to feeds and water *ad libitum*. Prior to and throughout this study, the mice were humanely handled under good laboratory practices and ethics.

*2.3 Experimental design*

*2.3.1 Acute toxicity testing*

Twelve female adult mice (22.0 ± 0.5 g) were used for the acute toxicity testing. Based on the long/widespread use of and previous high acute safety reports on the selected medicinal plants [25; 27; 45; 46; 58; 60; 68], and to minimize the number of animals used, mice (n=3) were each administered an oral limit dose of an extract at 5000 g/kg/10 ml distilled water and 10 ml/kg distilled water for the control group. They were all observed acutely for toxicological signs for 24 hours, and thereafter, daily for 14 days.

*2.32 Behavioural studies*

The screening for the acute anxiolytic activities of the selected leaf extracts was carried out in randomized groups of mice (n=6; an equal number of sexes) weighing 21.0 ± 0.8 g in units of 40 cm square plexiglass open-field apparatus whose floors were each partitioned with gridlines into 16 equal squares. The behavioural experimentation took place in a quiet, fairly large room having about 10-lux illumination. Activity screening was done by placing a mouse orally administered 1 hour earlier with 125, 250, and 500 mg/kg extracts, 0.5 mg/kg diazepam or 10 ml/kg distilled water, in the centre of the open-field and allowed to freely explore the test arena for 5 minutes according to the procedures as described in [69] – with minor modifications. The innermost central squares were taken as the centre zone and the rest of the squares as the periphery of the test arena. Three important mouse anxiety parameters such as percentage centre zone time; which is the percentage of the test spent by the mouse with all four paws within the central zone, centre zone re-entries and frequency of defecation/urination by the animals were all observed and recorded

## 3. RESULTS AND DISCUSSION

*3.1 Plant extraction yields*

Table 1 indicates the extract yields of 21 g of *B. pinnatum*, and 40 g each of *T. dodoneifolius* and *T. catappa* dry leaf powders.

Table 1: Percent plant extract yields

| Extract | Yields (g) | %yield (g/100 g) |
|---|---|---|
| *Tapinnatus dodoneifolius* | 4.40 (bright green) | 20.10 |
| *Bryophyllum pinnatum* | 7.80 (deep green) | 19.50 |
| *Terminalia catappa* | 13.40 (rich brown) | 31.50 |

*3.2 Acute toxicity testing results*

The three plants appear to be well tolerated as the experimental animals exhibited no toxicological signs during the 24 hours following extract administration and throughout the 14-day test.

*3.3 Behavioural experiment results*

3.3.1 Effect of single acute doses of hydroalcoholic *B. pinnatum*, *T. dodoneifolius* and *T. catappa* leaf extracts on mean centre zone times (M%CZT) of mice (n+6) exposed to the OFT.

In the open field test (Table 2), compared to distilled water treatment, all the extracts caused a dose-dependent increases in the mean %CZT spent by the mice at the centre zones of the open field maze with a significant increase ($P<0.05$) in this rodent parameter at the highest doses of the extracts. The mean %CZT of 20.94±3.91 recorded by the experimental group treated with the highest dose of *T.dodoneifolius* was comparable to 22.61±1.31 recorded for the 0.5 mg/kg diazepam treated-mice.

Table 2: Effect of plant extracts on mean percent centre zone times

| Treatments (mg/kg) | Mean percent centre zone times (M%CZTs) | | |
|---|---|---|---|
| Plant extracts | 125.00 | 250.00 | 500.00 |
| D/water (10 ml/kg) | 6.39±1.53 | 6.39±1.53 | 6.39±1.53 |
| *T. dodoneifolius* | 7.50±1.73 | 13.72±2.43 | 20.94±3.91* |
| *B. pinnatum* | 9.72±1.91 | 9.78±1.32 | 19.67±2.01* |
| *T. catappa* | 6.95±1.27 | 12.17±2.01 | 16.84±1.49* |
| Diazepam (0.5) | 22.61±1.31* | 22.61±1.31* | 22.61±1.31* |

All results were expressed as mean ± S.E.M of mice (n=6). Analysis was done using the One-way ANOVA. Significance set at *P*-values ≤ 0.05.

3.3.2 Effect of single acute doses of hydroalcoholic *B. pinnatum*, *T. dodoneifolius* and *T. catappa* leaf extracts on the mean centre zone re-entries (MCZR) of mice (n+6) exposed to the OFT.

All the plant extracts (Table 3) caused dose-dependent increases in the mean frequencies of CZR but only the highest doses of *T. dodoneifolius and B. pinnatum* extracts caused significant (*P*-values ≤ 0.05) increases in the mean CZR that are comparable to that of 0.5 mg/kg diazepam treatment.

Table 3: Effect of plant extracts on mean centre zone re-entries

| Treatments (mg/kg) | Mean centre zone re-entries (MCZRs) | | |
|---|---|---|---|
| Plant extracts | 125.00 | 250.00 | 500.00 |
| D/water (10 ml/kg) | 4.67±1.15 | 4.67±1.15 | 4.67±1.15 |
| *T. dodoneifolius* | 7.33±0.88 | 9.50±0.76 | 11.50±1.6* |
| *B. pinnatum* | 6.00±1.15 | 10.50±0.76 | 12.50±1.72* |
| *T. catappa* | 8.17±1.60 | 10.17±1.67 | 10.83±1.96 |
| Diazepam (0.5) | 12.17±1.66* | 12.17±1.66* | 12.17±1.66* |

All results were expressed as mean ± S.E.M of mice (n=6). Analysis was done using the One-way ANOVA. Significance set at *P*-values ≤ 0. 05..

3.3.3 Effect of single acute doses of hydroalcoholic *B. pinnatum*, *T. dodoneifolius* and *T. catappa* leaf extracts on mean defecation/urination frequency (MD/UF) of mice (n+6) exposed to the OFT

Compared to the distilled water-treated mice, dose-dependent and significant ($p \leq 0.05$) mean D/UF reduction was only observed with *T. dodoneifolius and B. pinnatum* extracts-treated mice – with the two extracts at their highest doses of 500 mg/kg exhibiting significant ($P \leq 0.05$) mean D/UF reductions that even surpassed that of 0.5 mg diazepam treatment. On the other hand, *T. catappa*-treated mice exhibited dose-independent and insignificant ($P > 0.5$) reduction in this anxiety parameter.

Table 4: Effect of plant extracts on mean defecation/urination frequencies

| Treatments (mg/kg) | Mean defecation/urination frequencies (MD/UFs) | | |
|---|---|---|---|
| Plant extracts | 125.00 | 250.00 | 500.00 |
| Dist. water (10 ml/kg) | 4.83±0.75 | 4.83±0.75 | 4.83±0.75 |
| *T. dodoneifolius* | 4.17±0.48 | 3.17±0.54 | 1.83±0.60* |
| *B. pinnatum* | 3.17±0.79 | 2.33±0.42 | 1.33±0.49* |

| | | | |
|---|---|---|---|
| *T. catappa* | 2.83±0.60 | 2.00±0.73 | 3.67±0.42 |
| Diazepam (0.5) | 2.62±0.21* | 2.62±0.21* | 2.62±0.21* |

All results were expressed as mean ± S.E.M of mice (n=6). Analysis was done using the One-way ANOVA. Significance set at *P*-values ≤ 0. 05..

Extract yields of dry leaf powers of these medicinal plants in this study are relatively high (Table 1). *T. globiferus*, *B. pinnatum*, and *T. catappa* extracts have been associated with efficacy for several diseases in diverse traditional cultures [19; 42; 56], and the observed high extract yields of these medicinal plants may be an indication of the rich deposits of diverse bioactive phytoconstituents that have been reported for extracts obtained from these plants in previous studies [17; 20; 48; 50; 58; 60].

Despite previous reports of rich repository of phytochemicals in the extracts of these plants, the relatively high safety exhibited by these extracts are in agreement with some earlier reports of high tolerability for *T. dodoneifolius* DC. (Loranthaceae) [58; 61], *T. catappa* [67; 68] and *B. pinnatum* [70; 71] extracts. Their individual apparent wide margin of tolerability is well supported by the long and widespread ethnomedicinal uses of the three plants for the traditional alleviation of diverse diseases including infections, hypertension, diabetes mellitus, convulsions, cough, and cancers with scarce or no reports of significant toxicity [18; 19; 42; 52; 57].

OFT has remained one of the most relevant preclinical psychotropic drug discovery tools since its invention close to a century ago by Carl S. Hall to assess rodents' general locomotory and anxiety-related emotional behaviours [72; 73]. This test is based on the conflict created in the animal between its natural tendency to explore and aversion to exposure or open space when placed in the open-field maze [76; 77; 78]. This test has been deployed in the anxiolytic and sedative-anxiolytic activity screening of several medicinal plants in previous studies [80; 81; 82; 83]. Its attraction is viewed to derive from its operational simplicity, cost-effectiveness and relative high throughput when compared to other standard tests used in characterizing ethological patterns in animals and in the screening of anxiolytic or anxiogenic activity in both putative and established anxiety-modulatory agents [74; 75]. Its other strengths include the capacity to generate multiple anxiety-related parameters, and the affordability of temporal assessment of these parameters. High inter-parameter correlation and good predictive validity for full benzodiazepine and 5HT1A agonists [76; 77; 78] are some of its strong points. The plethora of rodent anxiety-related parameters that can be harvested on the OFT include percent centre zone time (proportion of test time spent in the central zone of field: the greater the percentage the less anxiogenic the animal is); centre zone entries (frequency of central zone entry and re-entries: higher values indicates anxiolysis); latency to centre zone entry (time delay before the animal enters the central zone of the maze, short latency indicates anxiolytic effect); defecation/urination frequency (higher faecal/ urine droppings indicate anxiogenicity) and the (instead of 'a') number of rears (a rodent event during which the animal stands erect on its hindlimbs with its

hindlimb either completely in the air (unsupported rear) or rests them against the walls (supported rear) of the maze – this parameter is viewed to mirror either anxiogenesis or anxiolysis depending if the rear is supported or not). Other parameters that can be obtained in the OFT are grooming (a rodent behavioural event in which the animal engages in self-cleaning, licking and brushing of face and body; it is said to indicate high anxiety level); stretch attend postures (the animal while lowering and keeping its trunk close to the floor of the test apparatus, alternately elongates and retracts it in order to assess the risks in its immediate environment – this parameter indicates anxiety); number of squares crossed (this is a measure of the locomotory and exploratory activities which is indicative of anxiolysis) and freezing (the animal pulls itself together and remains immobile usually close to the walls of the test apparatus – it indicates high anxiety levels) [76; 77; 78; 79].

The anxiolytic activity screening of hydroalcoholic *T. globiferus*, *B. pinnatum*, and *T. catappa* extracts in the OFT was assessed using the percent centre zone time, centre zone entries and defecation/urination frequency. On the mean percent centre zone time, all three plant extracts demonstrated dose-dependent anxiolytic activity that was significant at their highest doses and with the *T.dodoneifolius* extract's anxiolytic activity comparable to the standard anxiolytic drug. However, on the mean centre zone re-entries and mean defeaction/urination frequencies, only *T.dononeifolious* and *B.pinnatum* extracts exhibited dose-dependent anxiolytic effects that were significant and comparabele to those of 0.5 mg/kg of diazepam - the standard anxiolytic drug. This study is one of the few reports on the anxiolytic activity of these plants despite their widespread traditional use for the alleviation of broad spectrum of diseases [27; 32; 46; 47; 57; 63; 67]. These findings are in agreement with previous reports of similar anxiolytic, antidepressant and cognition-enhancing activities of *T.dodoneifolius* extracts [64] and anxiolytic activity of its congener *T.globiferus* extracts [65; 66] in mice activity. They are also similar to previous reports of anxiolytic activity of *B. pinnatum* and its congener *K. pinnata* extracts in young zebrafish and rodents [40; 84]. Anxiolytic activity reports on *T. catappa* were scanty but it has been reported to exhibit significant antidepressant effect in rodents subjected to chronic unpredictable stress [85].

Hydroalcoholic *T.dodoneifolius* and *B.pinnatum* extracts have demonstrated consistent anxiolysis across the three rodent anxiety parameters and *T. catappa* has less consistency in this study. This observation may be due to some of the limitations of this study. The first of these is the use of single acute as opposed to repeated acute doses of the extracts in the investigation of their anxiolytic activity. This is because some plant extracts exhibit delayed anxiolytic effects which single acute doses may be inadequate to produce. Secondly, the use of only the OFT which has been reported to be less sensitive to the anxiolytic activity of selective serotonin-reuptake inhibitors [86] and anxiety-related behaviours in some rodent genetic strains [87]) as a sole paradigm in this study, may have reduced the size and consistency of our findings.

## 4. CONCLUSION

The above findings justify the traditional uses of afore-mentioned plant extracts for the alleviation of nervousness and related disorders. However, further studies are recommended to fine tune the present findings.

## ETHICAL APPROVAL

Ethical approval for the conduct of this study was sought and obtained from the Ethical committee of the Ahmadu Bello University, Zaria.

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