
Original Research Article

Pharmacognostic standardization and insecticidal activity of the leaves of *Tecoma stans* Juss (Bignoniaceae)

Tavs A Abere* and Comfort O Enoghama

Department of Pharmacognosy, Faculty of Pharmacy, University of Benin, Benin City. PMB 1154, Nigeria

*For correspondence: Email: eseabere@yahoo.com Tel: +2348023395616

Abstract

Purpose: Many medicinal plants have been said to be protective against insect damage without any scientific proof that they possess insecticidal activity. This study was aimed at establishing the Pharmacognostic profile and insecticidal activity of the leaves of *Tecoma stans* Juss (Bignoniaceae) to confirm its traditional application and justify continuous usage.

Methods: Evaluation of the fresh, powdered and anatomical sections of the leaves were carried out to determine the macromorphological, micromorphological, chemomicroscopic, numerical and phytochemical profile. Evaluation of the insecticidal activity involved the determination of antifeedant properties, repellent and insecticidal actions of the extract and its fractions.

Results: Macro and microscopic studies gave results that could serve as a basis for proper identification, collection and investigation of the leaves of *T. stans*. Phytochemical screening revealed the presence of alkaloids, tannins, flavonoids, cardiac glycosides and saponins. The crude extract and fractions produced antifeedant, repellent and insecticidal activities to varying degrees.

Conclusion: Pharmacognostic parameters of *T. stans* that can aid its standardization and quality control has been provided. *T. stans* has the potential to act as a lead in the commercial production of insecticides of plant origin.

Keywords: Quality control, antifeedant properties, repellent action .

Indexing: Index Copernicus, African Index Medicus

Introduction

Insects cause the greatest losses in Agriculture e. g. Locust cause a lot of damage to agricultural products such as grains and cereals [1]. Several efforts have been made to control insects responsible for the transmission of diseases and causing damages to agricultural products. Some of the control strategies adopted include the use of synthetic chemicals either as larvicides targeting the larvae in their breeding sites or as insecticide killing the adult insects. Instead of the much expected results, the use of synthetic insecticides has led to the disruption of natural biological control systems, development of resistance and resurgence in insect populations. Most often the use of synthetic insecticides results in undesirable effects on non-target organisms with consequence environmental pollution. The adverse effects associated with the use of synthetic insecticides had led to the search for alternative methods of insect

control. Active agents derived from plant sources have been demonstrated to possess larvicidal, insecticidal, oviposition deterrent and repellency activities [2-7]. More so, these plant products have been claimed to be more ecological friendly than synthetic chemicals such as temephos, fenthion, diflubenzuron and methoprene used as both larvicides and insecticides. The insecticidal activity of ethanolic extracts of four tropical plants (*Vernonia amygalina*, *Sida acuta*, *Ocimum gratissimum* and *Telfaria occidentalis*) against beans weevils (*Acanthscelides obtectus*) has been established [8]. The laboratory evaluation of four medicinal plants as protectants against the maize weevil, *Sitophilus zeamais* (Mots) has also been investigated [9]. Insecticidal activity of powder and extracts of *Delonix regia* seed against maize weevil, *Sitophilus zeamais* (Coleoptera: Curculionidae) [10], evaluation of the powder of three medicinal botanicals in the control of maize weevil, *Sitophilus zeamais* Motschulsky [11] and insecticidal

and antifeedant activities of medicinal plant extracts against *Attagenus unicolor* Japonicus (Coleoptera: Dermestidae) [12] are other examples of insecticidal investigations of plant origin.

Phytochemical constituents such as alkaloids, tannins and glycosides from medicinal plants have been used extensively to control insect vectors due to their broad spectrum of activity, low mammalian toxicity and ability to degrade rapidly in the environment.

Bignoniaceae is a family of flowering plants comprising about 650-750 species in 116-120 genera. Members of the family have pinnately compound leaves. Simple leaves are rare and when observed are often dissected in pinnatifid or palmatifid fashion [13]. *Tecoma stans* can be found throughout the neotropical South Western United States, the Florida, Mexico, the Caribbean, the Bahamas, Central, South and Northern America and also in some parts of Africa, Philippines and Hawaii [14]. *T. stans* is commonly known as yellow bells (English), yellow trumpet bush (Afrikaans), esperazan (Spanish) and ododo ngbiligba (Ibo) [15].

The antimicrobial evaluation of the methanol extract of *T. stans* using a wide range of Gram-positive and Gram-negative bacteria and fungi showed remarkable broad spectrum of activity [16]. The genotoxic and cytotoxic study of the aqueous extract of *T. stans* showed no significant clastogenic effects *in vivo* but showed cytotoxic effects in the mouse embryo *in vitro* [16]. Antidiabetic investigations showed that the observed effects is due to the intestinal alpha-glucosidase inhibition by decreasing the postprandial hyper-glycaemic peak [17]. Phytochemical investigation of *T. stans* Juss fruits and flowers resulted in the isolation of a new phenylethanoid, 2-(3, 4-dihydroxyphenyl) ethyl-2-O-{6-deoxy-alpha-L-mannopyranosyl-4-(3,4-dihydroxyphenyl)-2-propenoate}-beta-D-glucopyranoside and a novel monoterpene alkaloid, 5-hydroxy-skytanthine hydrochloride, along with eleven known compounds, with most of them possessing strong scavenging activity to DPPH, peroxy and hydroxyl radicals [18]. The pharmacognostic investigations of the leaves of the Asian specie of *Tecoma stans* Linn. has been carried out [19]. The Indian researchers adopted the protocol established in our laboratory which was used to investigate the Pharmacognostic profile of *Mitracarpus scaber* Zucc [20] and *Dissotis rotundifolia* Triana [21]. Investigating the Pharmacognostic profile of the African specie of *T. stans* will determine if there are geographical variations. The result of the present study will not only aid in establishing the Pharmacognostic standardization of *T. stans*, but will also be useful in promoting research aimed at the development of new agents from medicinal plants for insect control.

Experimental

Preparation of plant extract

The leaves of *Tecoma stans* Juss (Bignoniaceae) were collected from a residential area of Ugbowo, Benin City, Edo State, Nigeria. The plants were authenticated by Mr. Sunday Nweke, the plant curator at the Herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, University of Benin, Benin City where voucher specimens were deposited. The fresh leaves were air-dried for 72 h and powdered using an electric mill.

Extraction and Partitioning

The dried leaves of *T. stans* (1.2 kg) were extracted using maceration method with MeOH (3 X 2L). Evaporating the solvent using rotary evaporator at 40 °C yielded an extract which was subsequently resuspended in water and successively partitioned into petroleum ether (3 X 2L), Chloroform (3 X 2L) and n-BuOH (3 X 2L). The fractions were concentrated *in vacuo* and used for insecticidal experiments.

Insect culture

Adult *Sitophilus zeamais* (maize weevil) and *Acanthscelides obtectus* (Beans weevil) used for the study were obtained from naturally infested maize and beans grains respectively in Uselu market, Benin City, Nigeria. From these stocks, pure cultures were raised to obtain new generations in the laboratory following standard procedure [22]. Freshly emerged adults were used for the experiments.

Macroscopy

The following macroscopic characters for the fresh leaves were noted: size and shape, colour, surfaces, venation, presence or absence of petiole, the apex, margin, base, lamina, texture, odour and taste [23 and 24].

Microscopy

The outer epidermal membranous layer (in fragments) were cleared in chloral hydrate, mounted with glycerin and observed under a compound microscope. The presence/absence of the following was observed: epidermal cells, stomata (type and distribution) and epidermal hairs (types of trichomes and distribution). The transverse sections of the fresh leaves through the lamina and the midrib as well as a small quantity of the powdered leaves were also cleared, mounted and observed [25].

Chemomicroscopic examination

Examination of the powder for starch grains, lignin, mucilage, calcium oxalate crystals, cutin and suberin were carried out using standard techniques [24].

Phytochemical investigation

Chemical tests were employed in the preliminary phytochemical screening for various secondary metabolites such as tannins (phenazone; iron complex; formaldehyde and modified iron complex tests were carried out on the aqueous extract to detect the presence of hydrolysable, condensed and pseudo tannins), cardiac glycosides (Keller - Killiani and Kedde tests were carried out on the methanolic extract to detect the presence of a deoxy sugar, whose natural occurrence is to date, known only in association with cardiac glycosides and to indicate the presence of a lactone ring on the cardenolides respectively), alkaloids (Mayer's, Dragendorff's, Wagner's and 1% picric acid reagents to detect the presence of alkaloidal salts and bases), saponins glycosides (frothing of the aqueous extract when shaken and haemolysis test on blood agar plates were carried out to indicate and confirm the presence of saponins), anthracene derivatives (Borntrager's test for combined and free anthraquinones, where aglycones were extracted using chloroform and shaken with dilute ammonia) and cyanogenetic glycosides (sodium picrate paper test were used to test for the presence of hydrocyanic acid in the sample. Conversion to sodium isopurpurate indicates the presence of cyanogenetic glycosides) [24, 26, 27 and 28].

Quantitative investigation

Quantitative investigations to determine moisture content, total ash, acid-insoluble ash, water-soluble ash, alcohol (90 % ethanol) and water soluble extractive values were carried out using standard procedures [25 and 29].

Moisture content

The powdered drug (2.0 g) was weighed into a clean crucible of known weight. After oven drying at 105 °C for 5 hours and cooled, the crucible was weighed again to determine weight loss in the powdered drug. The average percentage weight loss, with reference to the air dried powdered drug was determined for thirty replicates.

Total ash determination

The crucibles were washed thoroughly, dried in hot oven at 100 °C, cooled in desiccators and weighed. A 2.0 g portion of each of the samples were weighed into the crucible and put in the furnace. Heating was

started gradually until temperature of 600 °C was reached. This temperature was maintained for 6 hours. The crucible was then put inside the desiccators and cooled. After cooling the sample was reweighed and the percentage ash calculated. Thirty replicates were determined.

$$\% \text{ Ash} = \frac{(W - Z) \times 100}{N}$$

where W = weight of the crucible and ash, Z = weight of empty crucible, and N = weight of sample.

Acid-insoluble ash determination

The total ash was treated with 25 ml dilute hydrochloric acid in a crucible, boiled gently for 5 min while covered with a watch glass and filtered through ashless filter paper (Whatman No.1) of known weight. The crucible was washed with hot water and the washings passed through the filter paper. This was continued until the filtrate became neutral to litmus paper. The paper with the insoluble matter was dried to a constant weight at 105 °C. The weight of the insoluble matter was determined by subtracting the weight of the filter paper from the dry weight of the filter paper containing the insoluble ash. The percentage of the acid-insoluble ash with reference to the air-dried material was calculated. Fifteen replicates were determined.

Water-soluble ash determination

The water-soluble ash was determined by adding 25 ml of water to the ash. After boiling gently for 5 min, the content of the crucible was filtered through previously weighed dried ashless filter paper. After washing the residue with hot water, the filter paper was dried in an oven at 105 °C until a constant weight was obtained. The weight of the residue was obtained by subtracting the weight of the dry filter paper from the weight of the residue and the filter paper. The weight of the water soluble ash was then obtained by subtracting the weight of the insoluble ash (i.e. the residue) from the weight of the total ash. The percentage of water soluble ash with reference to the air-dried powdered material was then determined. Fifteen replicates were determined.

Alcohol soluble extractive value

Powdered leaf drug (5.0 g) was weighed into a 250 ml stopper conical flask. Ethanol 90 % (100 ml) was added to the conical flask and stoppered. The flask was shaken in a mechanical shaker for 6 hours and then allowed to stand for 18 hours. The extract was filtered by suction filtration using a Buckner funnel. The weight of a heated cooled flat bottom porcelain crucible was accurately determined. The filtrate was poured into weighed crucible and evaporated to dryness at 100 °C. The residue was dried to constant

weight and the final weight noted. The weight of the residue obtained from the extract was determined by subtracting the constant weight of crucible from the residue. The alcohol extractive was then calculated with reference to the initial weight of the powdered drug and expressed as percentage. Thirty replicates were determined.

Water soluble extractive value

The above experiment was repeated using water.

Insecticidal evaluation

The determinations of antifeedant and repellency activities were a modification of Khani *et al.*, [30] while that of the insecticidal activity is a modification of Arannilewa *et al.*, [9].

Determination of Anti-Feedant Properties

Beans weevils (20) were put into each of six (6) conical flasks containing a cube of sugar. Each cube was treated with 0.2 micrograms of the crude extract, fractions (Pet ether, chloroform, N-butanol and aqueous fractions) and distilled water respectively. The flasks were covered with a stopper and the movement of the insects noted for 5mins at every hour for the first 10 hours and at the end of 24th hour. The experiment was repeated using maize weevils.

Determination of Repellant Action

Beans weevils (20) were put into each of six (6) conical flasks. With the aid of a micro syringe, 0.2 microgram of the crude extract and fractions (Pet ether, chloroform, N-butanol and aqueous fractions) dissolved in DMSO were put at the bottom of the flasks and DMSO (diluent) served as the control. The flasks were covered with a stopper and the movement of the insects noted for 5mins at every hour for the first 10 hours and at the end of 24th hour. The experiment was repeated using maize weevils.

Determination of Insecticidal Action

Beans weevils (20) were put into each of five (6) conical flasks. With the aid of a micro syringe, 0.2 microgram of the crude extract and fractions (Pet ether, chloroform, N-butanol and aqueous fractions) dissolved in DMSO were sprayed into the flasks respectively. DMSO served as the control.

The time of injection was noted and the conical flasks were covered to prevent the weevils from escaping. After twenty-four hours, the number of dead weevils were counted and the rate of kill determined. The experiment was repeated using maize weevils.

Results

Macroscopic Description of the leaves of *T. stans*

The leaves were dark green in the upper surface and light green in the lower surface. The upper surface was smooth while the lower surface was rough. The leaves were simple leaf with a soft texture, lanceolate shape, serrated margin, apex acuminate, base acute and venation was reticulate. Average leaf size was $10.31 \text{ cm} \pm 0.5$ (length) and $3.74 \text{ cm} \pm 0.4$ (breadth). The fresh leaf had a slightly bitter taste and no specific odour.

Microscopic Description of the leaves of *T. stans*

Micromorphological features revealed that anticlinal walls are straight. Stomata were present in both lower and upper epidermi. The stoma was surrounded by two subsidiary cells whose common wall was parallel to the long axis of the guard cells (Paracytic arrangement) (Fig. 1). There were numerous unicellular covering and glandular trichomes present on both surfaces.

A transverse section of the leaf across the mid-rib showed an upper and lower epidermi consisting of cells of similar sizes. It had an isobilateral arrangement i. e. the two surfaces were identical. The mesophyll consisted of an upper and lower palisade layers and a spongy mesophyll, embedding a crystal sheath. There were crystal clusters of calcium oxalate in the spongy mesophyll. The mid-rib bundle was surrounded by a zone of collenchyma on both surfaces. The phloem vessels embedded the xylem vessels.

Chemomicroscopic examination of the leaves revealed the presence of starch, mucilage, calcium oxalate crystals and cellulose.



Figure 1: Photomicrograph of *T. stans* showing straight-walled epidermal cells and paracytic stomata arrangement.

Numerical data of the leaves of *T. stans*

The moisture content of *T. stans* which fell within the Pharmacopoeia limit, the ash values as well as the amount of constituents which were extractable by methanol and water under specified conditions are presented in Table 1.

Table 1: Numerical data of leaves of *Tecoma stans*

Parameter	Mean \pm SEM (% w/w)
Moisture content*	11.75 \pm 0.26
Total ash*	12.81 \pm 0.74
Acid – insoluble ash†	1.54 \pm 0.23
Water – soluble ash†	7.72 \pm 0.41
Alcohol – soluble extractive*	13.45 \pm 0.62
Water – soluble extractive*	9.04 \pm 0.09

*n = 30, †n = 15

Phytochemical screening

Phytochemical screening of the leaves of *T. stans* for secondary plant metabolites revealed the presence of alkaloids, tannins, flavonoids, saponins and cardiac glycosides.

Insecticidal investigations

Antifeedant activity

Within the 24 hours observation period, there was

evidence of antifeedant activity as both the beans and maize weevils did not feed on the sugar source where the extracts and fractions were present, but fed on the sugar source with distilled water.

Repellant activity

Within the 24 hours observation period, there was evidence of repellant action as both the beans and maize weevils moved from the bottom of the flasks where the extracts and fractions were present, but remained at the bottom of flask in the case where DMSO was present.

Insecticidal activity

The crude extract (Table 2) of *Tecoma stans* as well as the fractions (Table 3) showed different rates of insecticidal activity.

Discussion

Standardization of herbal medicines is the process involving a series of experiments that reveal and assemble a set of inherently peculiar characteristics. Such characteristics include constant parameters, definitive, qualitative and quantitative values or specific, unique and unshared features on the basis of which similar herbal medicines, claimed to be the same, can be compared for the purpose of authenticity, genuineness, purity, efficacy, safety,

Table 2: Insecticidal activity of the crude extract of *T. stans*

Solvent system	Concentration (μ g)	No of weevils	Mortality after 24hrs	Mortality rate after 24 hrs (%)
Extract (Beans weevil)	0.1	20	07	35
	0.2	20	07	35
	0.3	20	09	45
	0.4	20	12	60
Extract (Maize weevils)	0.1	20	02	10
	0.2	20	05	25
	0.3	20	06	30
	0.4	20	07	35
DMSO	0.2	20	00	00

Table 3: Insecticidal activity of the fractions of *T. stans* extract against beans weevils

Fractions Beans weevils	Concentration (μ g)	No. of weevils	Mortality after 24hrs	Mortality rate after 24 hrs (%)
Aqueous	0.2	20	01	05
N-butanol	0.2	20	03	15
Chloroform	0.2	20	04	20
Petroleum ether	0.2	20	09	45
Maizeweevils				
Aqueous	0.2	20	01	05
N-butanol	0.2	20	02	10
Chloroform	0.2	20	02	10
Petroleum ether	0.2	20	05	25
DMSO	0.2	20	00	00

repeatability, reproducibility and the overall quality assurance [31]. The macro- and micro-morphological parameters described in this study could therefore, serve as a basis of proper identification, collection and investigation of the leaves of *Tecoma stans*. Whereas there were minor differences in macro-morphology between the Asian and African species of *T. stans*, there were major differences in the micro-morphology. In our report, the anticlinal walls are straight and stomata arrangement is paracytic compared to the Asian specie as reported by the Indian researchers where the anticlinal walls were wavy and stomata arrangement anisocytic [19].

The Pharmacological activities of a given plant are associated with the type and nature of secondary plant metabolites present. The need for phytochemical screening has become imperative, since many plants accumulate biologically active chemicals in their tissues. Phytochemical evaluation of *T. stans* revealed the presence of tannins, flavonoids, alkaloids, saponins and cardiac glycosides. These compounds detected in the plant are known to possess medicinal properties and health promoting effects.

The numerical data were determined to assist in establishing the identity of crude drugs. Not only is the purchase of drugs which contain excess water uneconomical, but also in conjunction with suitable temperature, moisture will lead to the activation of enzymes and given suitable conditions, to the proliferation of living organisms [24]. As most vegetable drugs contain all the essential food requirements for moulds, insects and mites, deterioration can be very rapid once infestation has taken place. The moisture content of *T. stans* obtained in the determination of quantitative standards met the pharmacopoeial limits of water content for vegetable drugs, which is between 8 – 14 % [25]. *T. stans* can be conveniently stored at room temperature without the deterioration of their active constituents.

When vegetable drugs are incinerated, they leave ash, which varies within fairly wide limits in many drugs and is therefore of little value for purposes of evaluation. In other cases, the total ash value is of importance and indicates to some extent the amount of care taken in the preparation of the drug [24]. The total ash usually consists of carbonates, phosphates, silicates and silica. In the determination of total ash values, the carbon must be removed at as low a temperature (450 °C) as possible because alkali chlorides, which may be volatile at high temperatures, would otherwise be lost. When the total ash was treated with dilute hydrochloric acid, the percentage of acid – insoluble ash was determined. This usually consists mainly of silica, as most of the natural ash is soluble leaving the silica as acid – insoluble ash which represents most of the ash from the contaminating soil [23 and 24]. A high acid-insoluble ash in drugs such as senna, cloves, valerian and

tragacanth indicates contamination with earthy material. Senna leaf, which may be used directly as the powdered drug, is required to have a low acid-insoluble ash (2.5 %) while hyoscyamus, which unavoidably attracts grit onto its sticky trichomes is allowed a higher value (12.0 %). *T. stans* with numerous trichomes and acid-insoluble ash value of 1.54 ± 0.23 % may therefore be used directly as powdered drugs. The acid-insoluble ash of 11.05 % [19] recorded for the Asian specie of *T. stans* is unrealistic as the total ash value recorded was 10.35 %. The acid-insoluble ash cannot be greater than the total ash value, since the former is a component of the latter.

Some insects damage crops by feeding on saps, leaves or fruits, and some are capable of transmitting disease to humans, pets and livestock. The development of safe and cost effective insecticides using indigenous plants is being investigated by researchers. However, many plant products have been said to be protective against insect damage without any scientific proof that they possess insecticidal activities. From the results of this study, the leaf extract of *Tecoma stans* has shown to possess insecticidal, antifeedant activities and repellent action. The insecticidal activity of the plant may be due to the presence of chemical constituents in the plants [32]. The reported sensitivity of the weevils demonstrated in this study may largely be due to synergistic effect of the secondary plant metabolites present in *T. stans*.

Conclusion

The results obtained from this study showed that *Tecoma stans* Juss possesses unique Pharmacognostic parameters that can aid its standardization and quality control. The plant has the potential to act as a lead in the commercial production of insecticides of plant origin.

Declarations

Acknowledgement

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Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them. TAA conceived and designed the study. CO collected the sample and

carried out most of the Pharmacognostic investigations as well as the insecticidal investigations.

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