ANTI-INFLAMMATORY AND ANTI-DEPRESSANT ACTIVITY OF THE METHANOL EXTRACT OF *TRICHOSANTHES CUCUMERINA* PLANT IN ALBINO WISTAR RATS

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Abstract: The bioactive constituents as well as the anti-inflammatory and anti-depressant activities of the methanol `extract of *Trichosanthes cucumerina* whole plant were evaluated to ascertain and validate its medicinal use. The bioactive constituents were qualitatively analyzed using standard methods while the formalin-induced paw edema model was used for the anti-inflammatory activity. The force swim test (FST) and tail suspension test (TST) were used for the anti-depressant activity. The yield of the crude extract was 5.76%. The qualitative analysis of the phyto-constituents revealed the presence of saponins, phenolic compounds, tannins, terpenoids, eugenols, flavonoids and alkaloids. Steroids were however not detected. The administration of graded doses (50, 100, and 200 mg/kg) of the extract to Albino Wistar rats in the FST showed anti-depressant activity by a significant ($p < 0.01$) reduction in the duration of immobility in the treated groups, at a dose of 50 mg/kg (96.40%), when compared with the control. The TST elicited a depression inhibition of 64.20% at a dose of 50 mg/kg, which closely compares with the inhibition produced by the standard, amitriptyline administered at 10 mg/kg. In the anti-inflammatory test, the paw thickness of the experimental animals reduced from the initial 3.03 mm to 2.83 mm (after 4 hours) at an optimum dose of 50 mg/kg which compared favourably with the reduction produced by the standard acetyl-salicylate. Therefore *T. cucumerina* is a pharmacologically and ethnomedically important plant.

Keywords: *Trichosanthes cucumerina,* phytochemicals, anti-inflammatory, anti-depressant, forced swim test, tail suspension test.

Introduction

Traditional medicine has played a pivotal role in the phytotherapy of a large number of sicknesses (Theodoridis *et al*., 2023). Various parts of plants such as leaves, stems, roots, fruits, seeds and flowers, are directly or indirectly used in the formulation of therapies used for the treatment and management of these ailments (Mukherjee et al., 2022; Okigbo *et al*., 2009). Therefore, it has become imperative to study how plants with bioactivity can serve as viable remedial agents to some of these ailments such as inflammation and depression because, unlike synthetic drugs, they provide very negligible or no side effects when used and can easily be accessed. Plants with medicinal value have been utilized in the delivery of healthcare since ancient times. Globally, there are evidence-based studies to substantiate the efficiency of medicinal plants, and some of the results from these studies have furnished insights into the synthesis of plant-based compounds with therapeutic characteristics (Dhama *et al*., 2014). An attempt has been made by the World Health Organization to have a compendium of all medicinal plants used globally, and more than 20,000 species have been listed (Palhares *et al*., 2015). A large number of medicinal plant parts are used as raw drugs and they possess numerous and distinguishable medicinal properties (Mahesh and Sathish, 2009). Plants have a great potential for producing new drugs used in traditional medicine to treat chronic and infectious diseases (Panda *et al*., 2009). Presently, there is an increase in the need for therapeutic agents that will serve as treatment for many health challenges. Despite this increase in demand for natural therapeutic agents, many plants have either been neglected or are being underutilized. The low attention given to these plants by mainstream agriculture has been attributed to a variety of cultural, agronomic, social, genetic and economic reasons. However, there seems to be an increase in the recognition of medicinal plants because of their potential roles in alleviating the risk of agricultural production systems and their nutritional quality (Padulosi *et al.*, 2013). A very large number of Nigerians rely on herbal plants in dealing with various acute and chronic diseases. The use of medicinal plants in Nigeria cuts across social and economic classes. Both educated and uneducated people use medicinal plants in Nigeria mainly because of their cultural and spiritual ties (Hassanpour *et al.*, 2011).

Trichosanthes cucumerina is a perennial climber that is commonly eaten as a vegetable. It is a member of the *cucubitaceae* family. It is also known as viper gourd, snake gourd, or long tomatoes. *Tricosanthes cucumerina* is a monoecious annual herb with 2–3-branched tendrils that can grow to be 5–6 meters long. The stems are thin, green, 4-angled, slightly hairy, and have a mildly disagreeable odour. The roots are tuberous and pale in colour. The leaves are 7 to 14 centimeters long and broad, and 3 or 5-lobed. (Adeboye, 2008).

Plate 1: Leaves of *Trichosanthes cucumerina*

Plate 2: *Trichosanthes cucumerina* plant with fruits

The fruit of the plant is largely eaten as a vegetable by many rural dwellers most particularly in the western region of Africa (Ademosun *et al*., 2013). The plant contains a high concentration of chemical constituents such as carotenoids, phenolic acids, and flavonoids making it pharmacologically and therapeutically active but it is grossly underutilized. However, *T. cucumerina* is gaining prominence in alternative and complementary medicines like Ayurveda and Siddha because of its numerous pharmacological activities like anti-inflammatory, hepatoprotective, larvicidal effects, antidiabetic (Sandhya *et al.*, 2010) as well as cytotoxicity against certain cancer lines (Kongtun *et al*., 2009).

This study is centered on the phytochemical screening, anti-inflammatory and antidepressant properties of *Trichosanthes cucumerina* whole plant extract to provide a scientific basis for its therapeutic applications.

Materials and Methods

Collection and identification of sample

Trichosanthes cucumerina plant was harvested whole (apart from the root) from a private garden at Ugbowo (6º23′11″N 5º36′42″E) in Benin City, Edo State, Nigeria. The plant was identified and authenticated at the Department of Plant Biology and Biotechnology, University of Benin. The voucher number UBH–T533 was assigned to the plant and a specimen was deposited at the herbarium of the Department.

Sample preparation

The plant sample was rinsed with clean water and air-dried in the laboratory (away from sunlight) for two weeks (14 days). The dried plant was pulverized to a fine powder using an all-steel electric blender. Thereafter, 200 g of the powdered plant was extracted by maceration in 1 Litre of methanol (96%) for 72 hours with intermittent stirring to allow for proper dissolution of soluble matter. The mixture was then filtered and allowed to gently evaporate over a water bath set at 40°C until a slurry was obtained as the crude extract. The yield was calculated and recorded. The extract was then carefully transferred into an air-tight container and stored in the refrigerator (at 4°C) for further analysis.

Experimental Animals

The experiment was carried out on albino Wistar rats, weighing between 180 and 250g, of both male and female sexes to study the antidepressant and anti-inflammatory activities of the methanol extract of *T. cucumerina* plant. The rats were obtained from the Department of Biochemistry, Animal House, University of Benin, Benin City, and Nigeria. They were housed in 12-hour light and dark cycles and fed with pelleted food and water. The animals were allowed to acclimatize to laboratory conditions for 2 weeks. The animals were properly handled according to protocols for Laboratory use of animals set by the Faculty of Life Sciences Ethical Committee. The ethical approval number LS21311 was given for the study.

Phytochemical screening of Tricosanthes cucumerina

The qualitative test for the secondary metabolites was carried out on the powder of *T. cucumerina* plant. Exactly 5 g of the sample was weighed into a 250 mL beaker and 30 mL of methanol was added. It was then allowed to be extracted for 30 minutes and filtered. Test for alkaloids, steroids, eugenols, terpenoids, tannins, flavonoids, saponins and phenolic compounds were carried out on the filtrate using the standard method described by Trease and Evans, (2002) with modifications by Aiwonegbe *et al*, (2019).

Antidepressant activity

Experimental protocol

The control group was treated with 0.2 mL of distilled water alone, while the standard drug group was administered with 10 mg/kg of amitriptyline. Treated groups received 50, 100 and 200 mg/kg per body weight of *T. cucumerina* methanol extract.

Despair Swim Test

The antidepressant activity was determined using the forced swim test (FST) standard protocol. During the processes, animals were individually dropped on top of water in a glass cylinder 20 cm high and 14 cm in diameter, filled with water up to 10 cm high at room temperature. The animals were subjected to force swimming for 300 seconds (5 minutes) and immobility duration was observed and measured for 300 seconds (5 minutes) intervals. Immobility time was observed as time spent by the rats floating in the water without effort but required to project their head above water level.

To ensure fitness level among the animals, a pre-test was conducted 24 hours before FST by subjecting each tested rat to a 15-minute swimming session (Furuzan *et al*., 2000).

Tail suspension test (TST)

Tail suspension test (TST) was carried out following the method described by Steru *et al*, (1985) and Häuser *et al*, (2012). Rats were suspended on a bench at a height of 58 cm from the floor with the aid of an adhesive tape, positioned around 1 cm from the tail tip. The total period of immobility was measured in the test phase for 300 seconds (5 minutes). Rats were regarded as immobile when entirely stationary.

Anti-inflammatory test: formalin-induced paw oedema

Male and female sexes of Albino Wistar rats were selected and put into five (5) groups with 3 animals in each group. The control group received 0.5 mL/kg per body weight of distilled water orally. The standard group received aspirin (100 mg/kg orally). The treated groups were orally given 50, 100 and 200 mg/kg of *T. cucumerina* extract. All drugs were administered 30 minutes before inducing acute inflammation by using a single sub-plantar injection at 0.1 mL solution of freshly prepared 6% (w/v) formalin suspension in distilled water using the method described by Agbaje *et al*, (2008) and modified by Joseph *et al*, (2009). Formalin suspension in distilled water was sub-plantarly injected into the left hind paw of rats in the tested groups while the right hind paw was injected for the control group.

Results and Discussion

Phytochemical Screening Trichosanthes cucumerina plant

The phytochemical screening of the methanol extract of the leaves and shoots of *Trichosanthes cucumerina* showed the presence of various bio-active compounds as displayed in Table 1.

Table 1: Qualitative phytochemical profile of methanol extract of *T. cucumerina* plant

Phytochemical	Inference
Saponins	+
Phenolic compounds	+
Flavonoids	+
Tannins	+
Terpenoids	+
Eugenols	+
Steroids	-
Alkaloids	+

Key: + = present and - = not detected

Saponins, phenolic compounds, tannins, terpenoids, eugenols and alkaloids were present whereas steroids were not detected.

Anti-inflammatory activity

The result obtained from the anti-inflammatory test of *T. cucumerina* plant extract is shown in Table 2. The methanol extract of *T. cucumerina* plant at doses of 50, 100 and 200 mg/kg reduced oedema significantly at the 3rd and 4th hours after the administration of the extract, when compared to the control group. The anti-inflammatory activity of the extract was found to be significant in inhibiting the formalin-induced rat paw oedema.

Table 2: Anti-inflammatory effect of *T. cucumerina* extract and acetylsalicylate on formalin-induced hind paw oedema in rats

		Paw thickness (mm)			
Treatment	**Doses (mg/kg)**	**1hr**	**2hrs**	**3hrs**	**4hr**
Control	DW	3.77±0.18[a]	3.93±0.1.9[a]	4.23±0.17[a]	4.33±0.23[a]
Acetylsalicylate	100	3.10±0.15[b]	3.13±0.09[b]	3.20±0.12[b]	3.23±0.12[a]
T. cucumerina	50	3.03±0.03[b]	3.00±0.06[c]	2.73±0.12[c]	2.83±0.18[c]
T. cucumerina	100	3.20±0.15[b]	3.20±0.17[b]	3.37±0.02[b]	3.37±0.38[a]
T. cucumerina	200	3.43±0.07[a]	3.77±0.03[a]	3.80±0.06[a]	3.60±0.06[a]

Values are mean ± SD; [a] = values not significant compared to the negative control; [b] = values for $p < 0.05$; [c] = values for $p < 0.01$; DW = distilled water

Anti-depressant activity

Tail suspension test (TST)

The methanol extract of *T. cucumerina* plant at the dose levels of 50, 100 and 200 mg/kg decreased the duration of immobility after administration of the extract as shown in Table 3. The extract produced a significant reduction ($p < 0.01$) in immobility time compared to the negative control. This level of significance was observed for all three doses used in the study. This implies that the anti-depressant ability of the methanol extract of *T. cucumerina* plant is not strictly dose-dependent.

Table 3: Anti-depressant effect of *T. cucumerina* methanol extract on rats in tail suspension test

Treatment	Doses (mg/kg)	Immobility (seconds)	% inhibition
Control	DW	122.00±12.70[a]	0.00
Amitryptiline	10	25.00±3.46[c]	79.50
T. cucumerina	50	43.67±3.38[c]	64.20
T. cucumerina	100	43.94±6.11[c]	64.00
T. cucumerina	200	45.67±3.67[c]	62.60

Values are mean ± SD; [a] = values not significant compared to the negative control; [b] = values for $p < 0.05$; [c] = values for $p < 0.01$; DW = distilled water

Force swim test (FST)

Table 4 shows the result of FST for Albino Wistar rats. The methanol extracts of *T. cucumerina* plant inhibited depression in the test animals to a highly significant level ($p < 0.01$) mainly at doses of 50mg/kg (96.40%) and 100 mg/kg (96.10%). There was a slight reduction in the anti-depressant activity (92.50%) for the animals treated with 200 mg/kg of the extract. This shows that the active principles in the extract are independent of the dose at which it is administered.

Table 4: Anti-depressant effect of *T. cucumerina* methanol extract on rats in force swim test

Treatment	Doses (mg/kg)	Immobility (seconds)	% inhibition
Control	DW	179.00±11.55[a]	0.00
Amitryptiline	10	9.33±1.63[c]	94.80
T. cucumerina	50	6.39±0.59[c]	96.40
T. cucumerina	100	7.00±3.61[c]	96.10
T. cucumerina	200	13.52±0.64[b]	92.50

Values are mean ± SD; [a] = values not significant compared to the negative control; [b] = values for $p < 0.05$; [c] = values for $p < 0.01$; DW = distilled water

Discussion

Plants have been used throughout history to cure human diseases. Worldwide, people still use medicinal plants for healing and relieving physical suffering. Many modern medicines have been derived either directly or indirectly from medicinal plants (Hosseinzadeh *et al.*, 2015). Many studies still focus on identifying new medicinal plants.

In this study, the phytochemical constituents of *T. cucumerina* plant extract were identified and their therapeutic value was assessed. Interestingly, the phytochemical analysis showed the presence of various important medicinal components in the tested plant, including alkaloids, saponins, phenol, flavonoids, tannins, eugenols and terpenoids. This result is comparable to those obtained for the leaves of *T. cucumerina* by Stellus and Nair (2015). The presence of these metabolites in the leaves and stems of the plant suggests that there is inherent potential in its usage as phytomedicines. The bulk of the phytochemicals produced by plants have proved to be effective in the treatment of numerous ailments that bedevil mankind.

The in vivo test used to evaluate the anti-inflammatory properties of *T. cucumerina* methanol extract shows that the extract has active principles against inflammation. At a dose of 50 mg/kg, the extract produced a significant ($p < 0.01$) reduction in paw inflammation. This action was sustained in incrementally throughout the period of the study. *T. cucumerina* has therefore proved to be a better anti-inflammatory agent than the standard drug, acetylsalicylate, which was only able to sustain anti-inflammatory action for one hour. Higher doses of the plant extract (100 and 200 mg/kg) were less effective in controlling inflammation in the experimental animals.

The FST and TST used for the antidepressant study show that the methanol extract of *T. cucumerina* is more effective at lower doses. At a dose of 50 mg/kg, the plant extract significantly ($p < 0.01$) reduced immobility time by 96.40% in the FST. But when 100 and 200 mg/kg of the extract were administered, 96.10% and 92.50% inhibition of depression were respectively obtained. The antidepressant activity of the methanol extract of *T. cucumerina* plant may be due to the type of secondary metabolites it contains. Studies have shown that alkaloids have analgesic and stimulant properties (Okeke *et al.*, 2023).

Conclusion

The present study has revealed the presence of different medicinal compounds in the leaves and shoots of *Trichosanthes cucumerina*. There seems to be a nexus between the photochemical constituents and the observed biological activity of the plant extract. The excellent antidepressant and anti-inflammatory activities of the plant extract on Albino Wistar rats show that *Trichosanthes cucumerina* might offer a novel promising therapy that will be beneficial for general health. However, there is a need to carry out more biological studies on the plant to determine the mechanism of action of the observed activities.

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