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Comprehensive Pharmacological Assessment and Phytochemical Analysis of *Tetrastigma angustifolia* (Roxb.) Deb.: Unveiling Analgesic, Anxiolytic and Hypoglycemic Potential in Traditional Medicinal Contexts

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Authors' contributions

This work was carried out in collaboration among all authors. Authors JMBQ, UTN and MU designed the experiments and conception. Authors JMBQ and UTN conducted the research work. Data interpretation and analysis were aided by author JMBQ. Authors AKN, CB, KFB, MS and MR wrote the manuscript and authors JMBQ, UTN and MU made the necessary corrections in the write up and gave final approval for the submission of revised version. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Despite its promising applications in traditional medicine, the bioactivity of *Tetrastigma angustifolia* and its constituent phytochemicals remain underexplored. This study sought to elucidate the potential therapeutic benefits of *Tetrastigma angustifolia* in animal models, specifically focusing on its analgesic, anxiolytic and hypoglycemic properties. Additionally, a comprehensive analysis of the plant's chemical composition was conducted.
Methodology: Methanolic extracts of *Tetrastigma angustifolia* leaves were subjected to phytochemical screening. The anxiolytic effects were assessed through the Elevated Plus Maze and Hole Board tests. Analgesic activity was evaluated using the Acetic Acid-Induced Writhing method, while hypoglycemic effects were determined through the Glucose Tolerance Test.
Results: Phytochemical analysis identified the presence of alkaloids (0.5%), tannins (1.2%), and flavonoids (0.8%). The extract demonstrated significant anxiolytic activity, with 60% increase in time spent in the open arms of the Elevated Plus Maze compared to control groups ($p < 0.01$). Analgesic properties were evident with a 50% reduction in writhing response ($p < 0.05$). Moreover, the extract exhibited dose-dependent hypoglycemic effects, resulting in a 30% reduction in blood glucose levels ($p < 0.001$) relative to controls.
Conclusion: *Tetrastigma angustifolia* exhibits considerable therapeutic potential as evidenced by its significant analgesic, anxiolytic and hypoglycemic effects. These findings suggest its potential utility in drug development. However, to substantiate the observed effects, further research is warranted, including *in silico* analyses and more sophisticated experimental validations.

Keywords: Tetrastigma angustifolia; bioactivity; phytochemical analysis; analgesic activity; anxiolytic activity; hypoglycemic activity.

1. INTRODUCTION

Therapies made from natural substances have been used for centuries because of their remarkable medicinal attributes that may cure complicated medical conditions with little to no side effects and at minimal expenditure. The various naturally occurring chemical components that are present in medicinal plants give rise to their therapeutic characteristics. Scientific study has advanced to the point where researchers can identify a variety of phytochemical substances in medicinal plants that are used for preventive objectives and have been shown to deliver diversified physiological actions [1]. Advances in technology and analytical techniques, such as metabolize and genome mining, have enabled the discovery of new drugs from medicinal plants, revealing their therapeutic potential [2].

Algesia, a different term of pain, is a vague, uncomfortable sensation brought on by a noxious stimulus that may originate either within or outside the body [3]. Pain can vary in intensity, quality, duration, and referral, and it is a personal, subjective response to a cause. Although it usually occurs as a direct result of tissue damage, it can also develop on its own or continue long after the wound has healed [4]. Currently, acetaminophen and non-steroidal anti-inflammatory drugs (NSAID), opioids and non-opioid analgesics, as well as additional medication classes like antidepressants and anticonvulsants and combinations of these, are available as treatments for pain [5]. Then anxiety and depression are prevalent psychological disorders affecting over 20% of adults, with their study becoming increasingly crucial in psychopharmacology [6, 7]. Strong feelings of dread, trepidation, and uncertainty brought on by anticipating a potentially dangerous situation or event are known as anxiety. These emotions frequently reach a level where they interfere with daily activities [8]. While psychotropic medications are a type of medication used to treat anxiety disorders, they are limited in their effectiveness due to adverse effects, dietary requirements, and drug interactions [9]. Researchers are exploring plant-derived chemicals to find new anxiolytic medications with fewer undesirable side effects due to the negative effects such as cognitive impairment, addiction, psychomotor impairment, disorientation, hostility, excitation, and anterograde amnesia from regular use of benzodiazepines [10]. Hyperglycemia is another major global health issue associated with diabetes mellitus. This can be attributed to an immediate or long-term insulin deficit, as well as cellular insulin resistance. The condition is linked to a reduced quality of life and increase risk

factors for death and injury [11]. The following are risk factors for diabetes mellitus, increased calorie intake, obesity, cardiovascular illness, excessive stress levels, and physical impairment [12]. Insulin and a variety of hypoglycemic medications, such as sulphonylureas, biguanides, α-glucosidase inhibitors, thiazolidinediones, and derivatives of meglitinide, are being used as therapies for the many forms of diabetes [13]. The adverse reactions to these treatments, as well as the requirement for daily subcutaneous injections in the case of insulin, suggest the need for novel and more potent medications. Conversely, several chemicals from plants that are utilized as anti-diabetics have been found and extracted [14].

Tetrastigma angustifolia (Roxb.) Deb. is a very crucial medicinal plant with numerous uses. It is an evergreen shrub belonging to the *Vitaceae* family. In India, Sri Lanka, Southern China, Thailand, and other Southeast Asian countries, this plant is very common [15]. In Bangladesh, this plant can be found in different hill tracts of Chittagong such as Hajarikhil, Bariadhala, Rangamati and so on. This plant is locally known as Nekung riubi [16].

Recent studies have revealed that *Tetrastigma angustifolia* (Roxb.) Deb. possesses a wide range of pharmacological properties. However, the number of *in-vivo* tests done on this plant is very few. So, our research study's main goals are to assess *Tetrastigma angustifolia* (Roxb.) Deb.'s phytochemicals and several pharmacological activities including analgesic, anxiolytic and hypoglycemic effects in animal models.

2. MATERIALS AND METHODS

2.1 Collection of Plant Materials

The plant material *Tetrastigma angustifolia* was collected for the current study on August 27, 2022, from the Hajarikhil Wildlife Sanctuary in Chattogram, Bangladesh. Dr. Bokhtear Uddin, a professor at the University of Chittagong's Department of Botany, identified the plant.

2.2 Preparation of Crude Extracts

The gathered plant material (leaves) was isolated from unwanted substances or plant components. Then, in the Phytochemical Research Laboratory of the Biological Faculty, University of Chittagong, they were cleaned, let to dry in the sun for two weeks, and crushed into fine powder. Until analysis, the powder was kept dry, cold, and dark. A clean round bottom flask (5 liters) containing around 800 grams of the powdered substance was filled with 2.8 liters of methanol. The container and its contents were covered with foil and stored for 15 days with occasional movement and swirling. Then the entire combination was filtered through a brand-new cotton plug and Whatman No. 1 filter paper. When the temperature of this filtrate combination was reduced using a Buchii Rota evaporator at 60°C, 24 grams of crude extract were obtained from the filter and kept in a refrigerated beaker.

2.3 Solvent-solvent Partition of Crude Extract

The Kupchan-modified Van Wagenen technique was used to divide the crude methanolic extract into solvent-solvent fractions using three distinct solvents: N-hexane, Dichloromethane, and Ethyl Acetate. [17].

2.4 Phytochemical Screening

Phytochemical investigations involved analyzing several chemical groups found in the extract. To detect phytochemicals such as carbohydrates, proteins, alkaloids, glycosides, flavonoids, phytosterols, saponins, tannins, terpenes, fats, phenols, and fixed oils, a tiny amount of crude methanolic extract from *Tetrastigma angustifolia* leaves was considered for initial quantitative phytochemical research using conventional procedures [18-20].

2.5 Experimental Animals

For the experiment, 4–5 weeks old Swiss albino mice (Mus musculus) of both sexes weighing 25–35 g each were employed. The experimental animals were procured from Rajshahi's ICDDRB supplier of laboratory animals. The animals were housed in fresh, dry polypropylene cages in the Animal House of the University of Chittagong's Pharmacy Department. The cages were adjusted to a 12-hour light-dark cycle, 25±2°C temperature, and 45-55% relative humidity. The mice were given an unlimited supply of water along with a typical laboratory diet. Food was not given to the mices for twelve hours before or throughout the trial. Because these animals are susceptible to changes in the surroundings, they are kept at the test site for at least three to four days before the experiment begins.

2.6 Study Design

To evaluate the *in vivo* activities of *Tetrastigma angustifolia*, mice were separated into eight groups for each activity, and five mice were studied in each group. For every activity, Group I was treated as a control (1% Tween 80 solution), Groups III & IV as methanol extracts of 400 mg/kg and 200 mg/kg, Groups V & VI as N-hexane samples of 400 mg/kg and 200 mg/kg, and Groups VII & VIII as ethyl acetate samples of 400 mg/kg and 200 mg/kg. But Group II is different in every activity, as it was standard. For anxiolytic activity, both the elevated plus maze method and the hole board test were evaluated with diazepam (1 mg/kg of body weight) as standard. On the other hand, the acetic acid-induced writhing method was used to evaluate the analgesic activity and diclofenac (50 mg/kg of body weight) was used as the standard group. Last but not least, a glucose tolerance test was employed to evaluate hypoglycemic activity, with glibenclamide (10 mg/kg of body weight) acting as the standard.

2.7 Evaluation of Anxiolytic Effect

2.7.1 Elevated plus maze (EPM) test

The elevated plus maze test is the most extensively used apparatus to assess the anxiety that depends upon the study of spontaneous behavior [21]. The test originated based on the relationship between exploration and fear [9]. In the plus-maze test, two pairs of identical platforms were fit out from a central platform (5 × 5 cm), were positioned facing each other, and were placed in two open arms (30 × 5 cm, enclosed by a 0.25 cm high border) and two closed arms (30 × 5 cm, encircled by 25 cm high walls). The device was raised 40 centimeters exceeding the ground. For this test, Diazepam (1 mg/Kg) used as the reference standard. All of the arms were open to the mice, and they were free to roam about and investigate the labyrinth for five minutes without any interference. Each mouse was properly weighed before each treatment and test samples' doses and control materials were modified accordingly. At every five minutes, the mice's open and closed arm entries and the duration of time consumed in every situation were noted. The maze was meticulously cleaned after every test. Later, the captured data was analyzed to conduct behavioral analysis.

2.7.2 Hole-board test

The Hole-board test is an additional method for determining the anxiolytic action of the experimental plant *Tetrastigma angustifolia*. The level of anxiety is determined by a test known as the hole-board test. The investigation was done using a piece of timber measuring 20 cm by 40 cm with sixteen equally positioned holes [22]. Head-dipping is the process by which an animal pokes its head into holes in the floor of an enclosed area using hole-board equipment. It is believed that measurements of neophilia, or purposeful exploration, may be acquired from the frequency and length of head-dipping, regardless of the animal's overall locomotors activity [23, 24]. Each of the mice was precisely measured before any medication, and the doses of the test samples and the control drugs were adjusted accordingly. In addition, Diazepam (1 mg/kg) served as the reference standard for this test. Mice were placed on a board and counted how many times they lowered their heads through eye holes during a five-minute trial session half an hour after treatment [21, 25].

2.8 Evaluation of Peripheral Analgesic Effect

2.8.1 Acetic acid-induced writhing method

The acetic acid-induced writhing method, a behavioral monitoring evaluation approach, measures mice's belly constrictions and hind limb stretching at the time of response when 0.7% of acetic acid is administered intraperitoneally. This technique involves giving the experimental animals intraperitoneal acetic acid injections to induce pain [26, 27]. As the procedure involves giving the animals in each group intraperitoneal injections of 0.7% acetic acid after giving them oral control, Diclofenac, and other test samples at zero hours. A forty-minute break is given between oral and intraperitoneal injections to guarantee adequate absorption. Mice are watched for fifteen minutes following the delivery of acetic acid to count squirms or writhing. When an animal begins to writhe but does not finish it, it is said to have completed full writhe and two-half writhing is regarded as one complete writhing. This facilitates comprehension of how oral and intraperitoneal delivery affects animal behavior.

The percentage protection against acetic acid was calculated using the following formula:

$$\% \text{ of inhibition} = \{(A - B) \times 100\} / A$$

Here,

A = No. of writhes in control group
B = No. of writhes in test group

2.9 Evaluation of Hypoglycemic Effect

2.9.1 Glucose tolerance test

The glucose tolerance test (GTT) is a widely accepted method for assessing hypoglycemic activity and detecting insulin resistance, diabetes, reactive hypoglycemia, and other carbohydrate metabolism issues by measuring the speed at which glucose leaves the blood through blood samples. Thirty starved animals were split into eight groups, each containing five mice, using this approach. Every group of mice was under proper observation. Following the administration of the extract for 30 minutes, 10% glucose solution (10 mg/kg body weight) was administered to each group. After glucose loading, blood specimens were drawn from the tail vein at 30-minutes, 90-minutes, and 120-minutes increments. Blood glucose levels have been assessed using a glucometer.

2.10 Statistical Analysis

Each study's results were shown as mean ± SEM (Standard Error of Mean). A one-way ANOVA and a post hoc Dunnett's t-test were used to examine the data using the statistical program Statistical Package for Social Science (SPSS, version 16.0). The p-values less than 0.05, 0.01, and 0.001 (*p<0.05, **p<0.01, ***p<0.001) were considered as statistically significant compared to the control. Microsoft excel has been used for the graphs.

3. RESULTS

3.1 Phytochemical Study

Phytochemicals, naturally present chemical compounds in plants, exhibit biological activity that protects plant cells against a range of environmental risks, including pollution, stress, UV exposure, and pathogenic assaults [28]. While they aren't essential nutrients necessary for human survival, they play crucial roles in disease prevention and management [29]. Phytochemical studies were represented by testing of separate chemical groups stated in the extract. The initial quantitative phytochemical analysis was conducted using a freshly made methanolic extract of *Tetrastigma angustifolia* leaves and we discovered some phytochemicals such as alkaloids, flavonoids, tannins, saponins, steroids, glycosides, cardiac glycosides, phenols, reducing sugar, and proteins using the standard methods (Table 1).

3.2 Evaluation of Anxiolytic Effect

3.2.1 Elevated plus maze (EPM) test

The elevated plus maze (EPM) serves as an *in vivo* method to assess the potential anxiolytic effects of substances. Typically, experimental animals exhibit a preference for enclosed areas of the maze, suggesting a natural aversion to open, unprotected spaces [30]. Out of all the fractions used in this experiment, the high dose methanolic extract significantly ($p < 0.001$) increased the number of entries (Fig. 1) in the elevated plus maze's arms and the time spent (Fig. 2) in the open arm compared to control, suggesting anxiolytic activity. Low-dose methanolic extract (200 mg/kg) also showed moderate (**P<0.01) time spent in open arms compared to control. In comparison to the groups treated with extract, there was a substantial ($p <0.001$) increase in both the number of entries and the time of stay in open arms after diazepam (1.0 mg/kg body weight) was administered (Table 2).

Table 1. Phytochemical screening of methanolic extracts of *Tetrastigma angustifolia* leaves

Serial No.	Phytochemicals	Test Name	Results
		Mayer's Test	
1	Alkaloid	Wagner's Test	+
2	Tannin	Ferric Chloride Test	+
		Salkowski's Test	
3	Steroid	Libermann-Burchard's test	+
4	Flavonoids	Alkaline Reagent Test	+
		Froth Test	
5	Saponins	Foam Test	+
6	Glycosides	Modified Borntrager's Test	+
7	Phenol	Ferric Chloride Test	+
8	Reducing sugar	Fehling's Test	_
9	Protein	Xanthoproteic Test	+
10	Cardiac glycosides	Keller Killiani Test	+

Note: The +/- sign indicates the presence/absence of the phyto constituents of META

Table 2. Data obtained from elevated plus maze (EPM) test

Group	(Mean ± SEM)			
	Open arm		Closed arm	
	Time spent (sec)	Number of entries	Time spent (sec)	Number of entries
Control	10.0± 1.3	6.8±0.73	272.21±4.1	11.2±0.86
Standard	79.4±3.35***	12.6±1.1***	205.8±4.5***	13.6±1.8***
ME-400	66.8±4.2***	8.4±0.67***	219.6±4.6***	14.6±1.02***
ME-200	44.6±2.8**	7.4± 0.81**	239.6±3.4**	11.2±0.86**
NH-400	28±2.3**	4.4±0.37	255.2±3.3*	7.6±0.68
NH-200	24.2±1.9	4.0±0.44	260.4±3.9	9±1.0
EA-400	26±3.7	3.8±0.37	257±5.4	6±1.0
EA-200	25.2±1.9	4.2±0.58	261±2.1	5.8±0.86

*Note: ME: Methanolic Extract, NH: N-hexane, EA: Ethyl Acetate. Results were expressed as Mean ± SEM; *P<0.05, **P<0.01, ***P<0.001 were considered statistically significant compared to control*

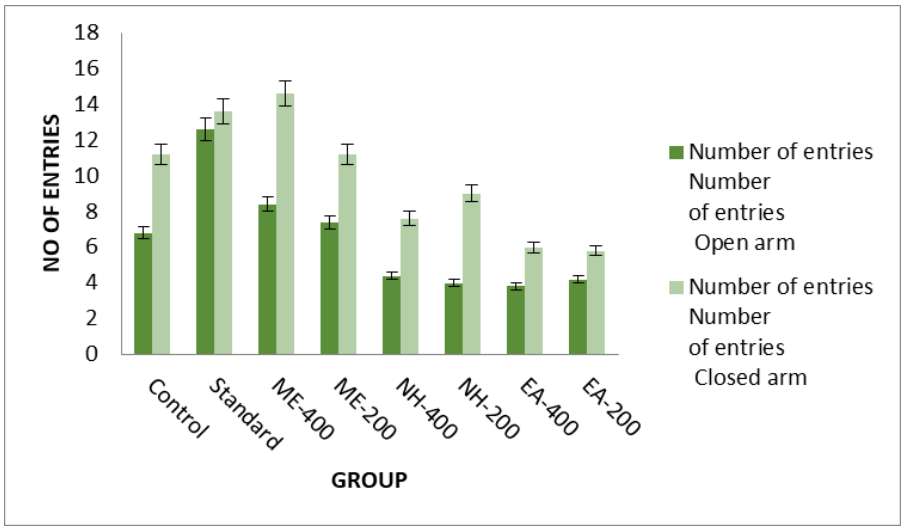

Fig. 1. Screening of anxiolytic activity of methanolic extract and soluble fractions of n-hexane and ethyl acetate of *Tetrastigma angustifolia* leaves by counting the mean number of entries in open and closed arm in EPM

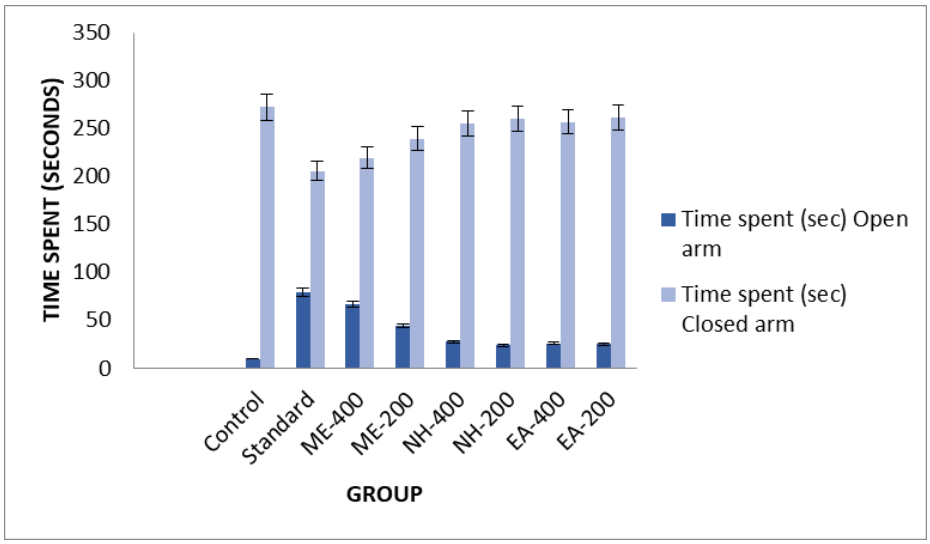

Fig. 2. Screening of anxiolytic activity of methanolic extract and soluble fractions of n-hexane and ethyl acetate of *Tetrastigma angustifolia* leaves by counting the mean time spent in open and closed arm of EPM

Table 3. Data obtained from hole-board experiment

Animal Group	Frequency of Dipping					Mean ± SEM
	M-1	M-2	M-3	M-4	M-5	
Control	36	32	25	26	20	27.8±2.8
Standard	75	81	73	80	72	77.4±1.4***
ME-400	52	43	48	41	40	44.8±2.3***
ME-200	23	31	29	25	24	26.4±1.5*
NH-400	34	31	39	28	40	34.4±2.2
NH-200	24	27	20	17	26	22.8±1.8
EA-400	28	22	17	21	26	22.8±1.9
EA-200	15	19	12	16	15	16±1.2

*Note: ME: Methanolic Extract. Results were expressed as Mean ± SEM; *P<0.05, **P<0.01, ***P<0.001 were considered statistically significant compared to control*

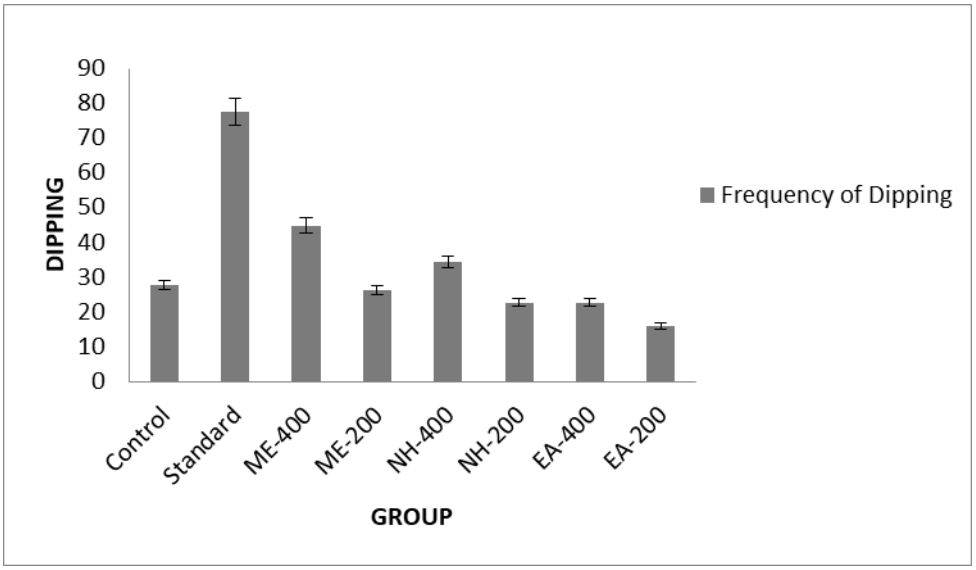

Fig. 3. Screening of anxiolytic activity of methanolic extract and soluble fractions of n-hexane and ethyl acetate of *Tetrastigma angustifolia* leaves by hole board method

3.2.2 Hole board test

The hole board test provides another perspective on assessing anxiolytic activity, focusing on the head-dipping behavior of experimental animals as the primary indicator. Sensitivity to alterations in emotional or anxiolytic states may lead to a rise in head-dipping habit during this test [23, 31]. The head dipping habit shown in the hole board test in Fig. 3 refer to changes to the emotional state of the experimental animal and suggest an expression of anxiolytic activity. The more head dipping into the hole counted as high anxiolytic activity. In this study, the high dose of methanolic extract (400 mg/kg) of the plant reported significantly high anxiolytic activity (44.8%) compared to control where the standard increased head dipping by about 77.4% (Table 3).

3.3 Evaluation of Analgesic Effect

3.3.1 Acetic acid-induced writhing method

An effective method for determining peripheral analgesic efficacy is the acetic acid induction assay [32]. Through chemo sensitive nociceptors, acetic acid causes a writhing reaction in experimental animals [33]. *Tetrastigma angustifolia*'s extract of methanol reduced writhes by 60.1% (**P<0.01) and 46.02% (**P<0.05) at doses of 400 and 200 mg/kg body weight, respectively, compared to the control group. This was identical to the standard drug Diclofenac Sodium's 76.9% inhibition (**P<0.001) (Fig. 4). The N-hexane soluble fraction showed the most significant inhibition with 72.7% (***P<0.001), and 53.8% (**P<0.01) at the dose of 400 and 200 mg/kg body weight. The ethyl acetate fractions also

showed moderate inhibition with 44%, and 33.9% at the dose of 400 and 200 mg/kg body weight, respectively (Table 4). During the writhing test, prostanoids and lipoxygenase products were shown to raise the peritoneal fluid levels dramatically. The extract's ability to inhibit acetic acid-induced writhing in mice shows analgesic properties. Flavonoids can disrupt eicosanoid synthesis and diminish arachidonic acid production by inhibiting neutrophil degranulation. Alkaloids are known to reduce pain perception. The pyridine ring of alkaloids has potent anti-inflammatory and ant nociceptive properties.

3.4 Evaluation of Hypoglycemic Effect

3.4.1 Glucose tolerance test

Enhancement of glucose tolerance in diabetic or insulin-dependent treated mice confirmed the hypoglycemic characteristic of the plant sample, suggesting that any plant sample might repair reduced glucose tolerance of diabetes and hence display an antidiabetic function [34]. During the test, after 30 minutes of glucose loading, the crude methanolic extract, n-hexane soluble fraction, and ethyl acetate soluble fraction significantly reduced blood glucose levels compared to the control in Figs. 5 and 6, which was comparable to the standard drug Glibenclamide. It was observed that the extract at both 400 mg/kg (***P<0.001) and 200 mg/kg (**P<0.01) doses significantly reduced blood glucose levels compared to control but 400mg/kg dose showed higher hypoglycemic activity. The N-hexane soluble fraction also showed significant hypoglycemic activity at both 400 mg/kg (***P<0.001) and 200 mg/kg (**P<0.01) doses. The ethyl acetate soluble fraction shows moderate hypoglycemic activity at both doses (*P<0.05) in (Table 5). The phytochemical screening of the extract revealed that it contained phytoconstituents such as alkaloids, flavonoids, tannins, and saponins. These elements can induce extraordinary antidiabetic action in medicinal plants, which may explain why these plants have been traditionally used to treat diabetes. More study is needed to identify the chemical responsible for this activity and develop it as a possible hypoglycemic medication.

Table 4. Data obtained from acetic acid-induced writhing experiment

Animal Group	Number of Writhing (Mean ± SEM)	% of Inhibition of Writhing
Control	55.4± 1.28841	0
Standard	12.8± 1.32***	76.9
ME 400	22.1± 3.42**	60.1
ME 200	29.9± 1.8*	46.02
NH-400	15.1±.90***	72.7
NH-200	25.6± 2.46**	53.8
EA-400	31.0± 2.23	44
EA-200	36.6± 1.91	33.9

*Note: Each value represents the mean ± SEM. (n= 5). One-way ANOVA followed by Dunnett's t-test. ** P<0.001, * P<0.05 compared with control. ME = Methanolic Extract and EA= Ethyl acetate*

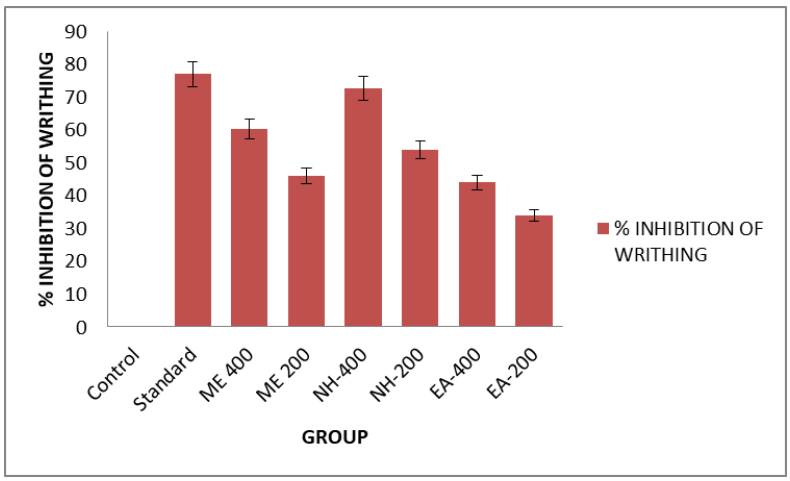

Fig. 4. Determination of analgesic activity of methanolic extract and its different fractions of *Tetrastigma angustifolia*

Table 5. Hypoglycemic activity of methanolic extract and its different fractions of *Tetrastigma angustifolia*.

Animal Group	Dose Mg/kg	Mean ± SEM			
		0 minute	30 minute	60 minute	120 minute
Control	0.15 ml/10 gm of body weight	6.3 ± 0.20	14.2 ± 1.3	11.49 ± 1.3	7.1 ± 0.66
Glibenclamide	10	4.9 ± 0.30	7.8 ± 0.44**	4.50 ± 0.21***	3.5 ± 0.18***
ME	400	6.03 ± 0.22	9.7 ± 0.48*	6.9 ± 0.69***	4.04 ± 0.40***
ME	200	6.0 ± 0.25	10.2 ± 0.43	7.4 ± 0.48*	4.64 ± 0.29**
N-hexane	400	4.5 ± 0.58	9.3 ± 0.43**	7.1 ± 0.63***	4.7 ± 0.33***
N-hexane	200	4.2 ± 0.411	10.4 ± 0.65	6.1 ± 0.16*	4.4 ± 0.21**
Ethyl acetate	400	4.9 ± 0.76	9.7 ± 0.85	6.0 ± 0.39	4.2 ± 0.11*
Ethyl acetate	200	5.5 ± 0.49	11.03 ±1.1	8.6 ± 0.81	5.5 ± 0.42*

*Note: Each value represents the mean ± SEM., N= 5. *P<0.05, **P<0.01, ***P<0.001 compared to the control, Dunnett's t-test after analysis. ME= Methanolic crude extract of leaves of Tetrastigma angustifolia, NH= N-hexane fraction of methanolic extract, EA= Ethyl acetate fraction of methanolic extract*

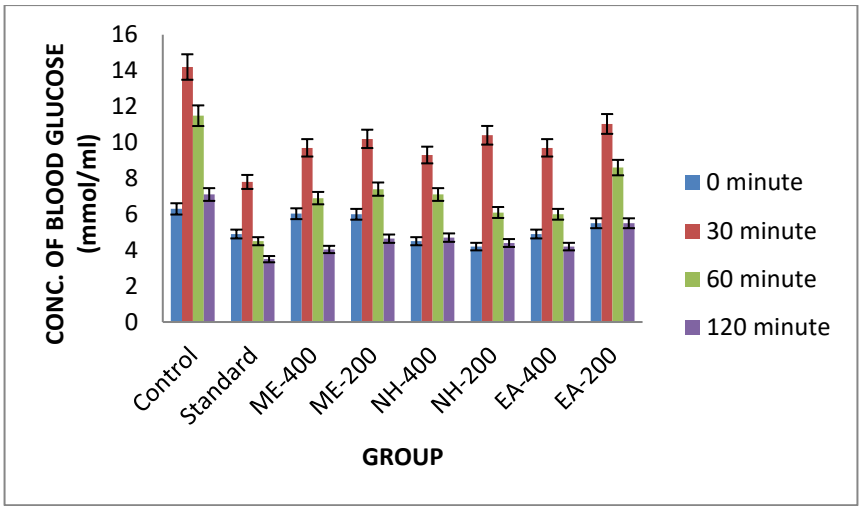

Fig. 5. Concentration of glucose in blood showed by methanolic extract and different fractions of *Tetrastigma angustifolia* at different times

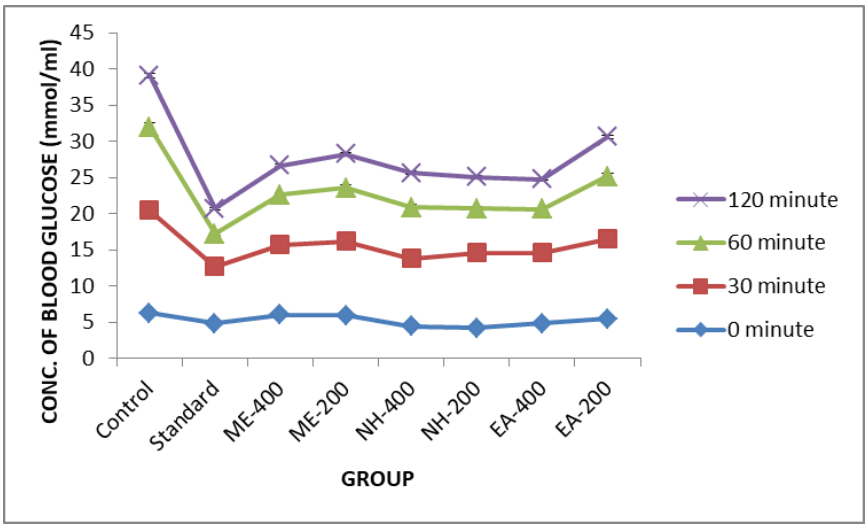

Fig. 6. Curves of concentration of glucose in blood showed by methanolic extract and different fractions of *Tetrastigma angustifolia* at different times

4. DISCUSSION

This study provides compelling evidence for the therapeutic potential of *Tetrastigma angustifolia*, demonstrating significant anxiolytic, analgesic, and hypoglycemic effects through a variety of *in vivo* assays. The elevated plus maze (EPM) and hole board tests, widely recognized for assessing anxiolytic activity, revealed that the high-dose methanolic extract of *Tetrastigma angustifolia* markedly increased both the number of entries and the time spent in the open arms of the maze. These observations suggest a pronounced anxiolytic effect, supported by statistical significance ($p < 0.001$) in comparison to the control group. Notably, the low-dose extract (200 mg/kg) also displayed a moderate increase in time spent in the open arms ($p < 0.01$), indicating potential therapeutic efficacy even at lower concentrations.

The hole board test further corroborated these findings, where the high-dose methanolic extract (400 mg/kg) resulted in a significant increase in head-dipping behavior, a behavior correlated with anxiolytic effects. The extent of this increase (44.8%) approached but did not surpass the anxiolytic response elicited by diazepam (77.4%), highlighting the substantial, though not yet fully comparable, and efficacy of *Tetrastigma angustifolia* in modulating anxiety-like behaviors.

The analgesic efficacy of *Tetrastigma angustifolia* was evaluated using the acetic acid-induced writhing test, a model known for its sensitivity to analgesic compounds. The methanolic extract demonstrated significant inhibition of writhing, with reductions of 60.1% and 46.02% at doses of 400 mg/kg and 200 mg/kg, respectively, compared to controls. This efficacy is comparable to Diclofenac Sodium, a standard analgesic, which exhibited a 76.9% inhibition. The n-hexane soluble fraction showed the most potent inhibition (72.7% at 400 mg/kg) and the ethyl acetate fraction displayed moderate inhibition (44% at 400 mg/kg), further reinforcing the plant’s analgesic potential.

Phytochemical screening revealed the presence of alkaloids, flavonoids, tannins, and saponins, all of which have been implicated in anti-inflammatory and analgesic activities. The anti-inflammatory properties of flavonoids, which can inhibit eicosanoid synthesis and arachidonic acid production, and the antinociceptive effects of alkaloids, particularly those with a pyridine ring, may contribute to the observed analgesic effects. These compounds likely interact with nociceptive pathways, providing a mechanistic basis for the observed reductions in pain responses.

The hypoglycemic activity of *Tetrastigma angustifolia* was confirmed through glucose tolerance tests in diabetic or insulin-dependent mice. Both the crude methanolic extract and its fractions (n-hexane and ethyl acetate) significantly reduced blood glucose levels, with the high-dose methanolic extract showing the most pronounced effect ($p < 0.001$). This activity was comparable to that of the standard antidiabetic drug, Glibenclamide. The observed dose-dependent reduction in glucose levels, especially at 400 mg/kg, underscores the potential of *Tetrastigma angustifolia* as an antidiabetic agent. The presence of alkaloids, flavonoids, tannins, and saponins in the extract aligns with the plant's traditional use in managing diabetes, as these phytoconstituents are known for their antidiabetic properties.

5. CONCLUSION

In conclusion, *Tetrastigma angustifolia* demonstrates considerable promise as a multi-faceted therapeutic agent with significant anxiolytic, analgesic, and hypoglycemic properties. However, the complexity of its bioactive components necessitates further research to isolate and characterize the specific compounds responsible for these effects. Comprehensive studies, including detailed mechanistic investigations and clinical trials, are essential to fully validate the plant's therapeutic potential and facilitate its development into a viable treatment option.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

ETHICAL APPROVAL

All authors officially affirm that the "Principle of Laboratory Animal Care" (NIH publication No. 85-23, revised 1985) and any applicable local or national legislation were adhered to. All experiments have been examined and approved by the appropriate ethics committee.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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