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SHORT COMMUNICATION article

Evaluation of antimicrobial, antioxidant, antidiabetic activities, and acute toxicity of *Elephantopus scaber* L.

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***Keywords*:** DPPH, *Elephantopus scaber* L., glipizide, medicinal plant, phytochemical properties

Abstract: *Elephantopus scaber* L., a medicinal plant from Myanmar, was studied to evaluate its biological activity. The activity of antimicrobial assessment showed that ethyl acetate extract exhibited moderate activity in contrast to all six tested organisms. Using the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay with ascorbic acid as standard for antioxidant activity showed that ethyl acetate extract possessed 36.28 µg/mL. The *in vivo* antidiabetic activity with glipizide, as a standard hypoglycemic agent was employed to determine antidiabetic activity. The data parameter under observation for *Elephantopus scaber* L. reaches its peak at 135 min, with a 39.0% reduction. No toxic behavior was observed at the limited tested concentrations (2000 mg/kg and 5000 mg/kg) and it suggested a favorable safety profile.

Introduction

Humans have gradually discovered natural phenomena in a widespread range. Due to this interest, man concerning his natural world is evident in his earliest records. Human activity in these records often reveals creative responses to natural phenomena. To this day, they have come closer to recognizing their limitations in fully understanding scientific phenomena and understanding the consequences of human activity on nature [1, 2]. Human life in all aspects has been greatly innovative through an ever-growing and powerful understanding of the natural world. However, it would be agreed that humans' relationship with natural resources over the years has not only established their rate but also has led to a greater collective awareness of how to more successfully tap into these enormous arrays of resources to additional human civilization. It might be argued that every knowledge system is somehow connected to or has sought inspiration from products of the natural world which are referred to as natural products [3]. Numerous rewards depended on the study of natural products. The discovery of a variety of useful drugs for the treatment of various diseases has led to and contributed to the development of science and technology and spectroscopic methods of structure elucidation. It has become one of the most important areas of application of natural products for the treatment of human diseases. In the cases of healthcare and medically-related scientific advances, nature has served as a crucial platform in facilitating advances in the field [2, 4, 5]. Natural products from medicinal plants, especially in pure compounds or standardized extracts,

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provide unlimited opportunities for the new drug. These findings led to the supreme availability of chemical diversity. With the increasing demand for chemical diversity in screening programs, and seeking therapeutic drugs from natural products, the more interest particularly in edible plants has grown throughout the world. Botanicals and herbal preparations for medicinal usage contain various types of bioactive compounds [6-9].

Elephantopus scaber L., commonly known as Elephant's Foot, is a versatile and intriguing plant that belongs to the Asteraceae family. Widely distributed across tropical and subtropical regions, this herbaceous perennial has been a subject of fascination for botanists, herbalists, and researchers alike. The scientific nomenclature, *Elephantopus scaber* L., reflects its distinctive characteristics, and the plant has earned various vernacular names in different regions, such as Elephant's Foot Plant, Prickly-leaved Elephant's Foot, and False Tobacco [10, 11]. *Elephantopus scaber* L. exhibits a wide geographical distribution, thriving in diverse ecosystems. Native to tropical and subtropical regions of Asia (India, Nepal, Pakistan, Sri Lanka, China, Taiwan, Hong Kong, Japan, Malaysia, Indonesia, Vietnam, Philippines, Thailand and Myanmar), the Americas (Neotropical regions extending from Southern Mexico through Central America and Northern South America to Southern Brazil), Africa and Europe, the plant has adapted to various climatic conditions. Its ability to grow in different soil types, from sandy to loamy, makes it a resilient species that can be found in both cultivated fields and uncultivated landscapes [12-14]. The novelty of this research lies in its integrative approach, systematically assessing multiple pharmacological properties within a single study. While prior research has explored its ethnobotanical significance, a unified evaluation of its therapeutic potential is lacking. This study fills that gap by providing scientific validation, offering valuable insights into modern pharmacology. The findings may contribute to future drug development and highlight the importance of natural products in contemporary medicine. The prominent pharmacological properties of the selected medicinal plant have prompted many phytochemical studies. Therefore, the plant has aimed to continue to evaluate its different pharmacological activities such as antimicrobial, antioxidant, antidiabetic, and acute oral toxicity in the present study. The study of medicinal plants for pharmacological applications is crucial for developing novel therapeutic agents. *Elephantopus scaber* L. is traditionally known for its bioactive properties, but comprehensive scientific validation remains limited. This study addresses the growing interest in plant-based therapeutics by evaluating its antimicrobial, antioxidant, and antidiabetic potential, along with acute toxicity for safety assessment.

Materials and methods

The chemicals used were purchased without further purification. The plant sample of *Elephantopus scaber* L. was collected from Mohnyin Township, Kachin State, Myanmar, and identified by the Department of Botany, Mohnyin University. The plant samples were sliced into small pieces and allowed to be air-dried at room temperature for about three weeks. These dried pieces of the sample were stored in a glass bottle with a stopper and used throughout the experiment.

Phytochemical screening: The preliminary qualitative phytochemical screening for the detection of different phytoconstituents such as alkaloids, carbohydrates, flavonoids, glycosides, gums and mucilage, phenolic compounds, polyphenols, quinones, reducing sugars, saponins, steroids, tannins, and terpenes were summarized by using standard methods in **Table 1**.

Antimicrobial activity: This study was carried out to evaluate the antimicrobial activities of the plant *Elephantopus scaber* L. with various solvents (n-hexane, ethyl acetate, and methanol) by using paper disc diffusion methods at the Department of Chemistry, Pakokku University.

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Antioxidant activity: The antioxidant activities of the various solvent extracts of the sample were determined by the DPPH free radical scavenging assay. The control solution was prepared by mixing 2.0 mL of 60 μM DPPH solution and 2.0 mL of methanol. The test solutions were also prepared by mixing 2.0 mL of the test sample solution and 2.0 mL of 60 μM DPPH solution with various concentrations. The blank solution was also prepared by mixing 2.0 mL of the test sample solution and 2.0 mL of 95.0% methanol. Moreover, these solutions were allowed to stand at room temperature for 30 min. Then the absorbance of each solution was measured at 517 nm by using a UV-2550 spectrophotometer.

Antidiabetic activity: In vivo antidiabetic (hypoglycaemic) activity, glipizide (a standard hypoglycemic agent) as a positive control for inhibiting the glucose level was used. The evaluation of the activity was carried out with the approval of the Department of Biotechnology, Mandalay Technological University, Ministry of Science and Technology. Mice (*Mus musculus*) with 30.0 g of body weight were used and kept separately from the others. Mice were divided into three groups, tested plant sample, positive control, and negative control. Each group contained five mice and gave them markers by using sodium picrate solution. Mice were prepared to cause hypoglycemic effects by given adrenaline injections. For adrenaline injection, mice were fasted overnight for 16-18 hrs, and injected intraperitoneally with adrenaline 0.2 ml/kg body weight as 6.0% solution in distilled water. Mice were starved for four hours after injection and then they were given 5.0 cc of 10.0% glucose solution orally at hourly intervals to prevent hypoglycaemic shock. They were offered unlimited amounts of standard laboratory diet food and water. After one week, the mice were used to test the hypoglycemic activity [15, 16].

Acute toxicity: The oral toxicity of the methanol extract of the plant *Elephantopus scaber* L. was tested by using the Limit Test of OECD guidelines (425), in *vivo* mice model at the Department of Biotechnology, Mandalay Technological University, Ministry of Science and Technology with an approval from the ethics committee of the University (2024).

Results and discussion

Preliminary phytochemical screening: Phytochemical screening of the plant of *Elephantopus scaber* L. showed the presence of different chemical constituents such as carbohydrates, flavonoids, glycosides, gums and mucilage, phenolic compounds, polyphenols, quinones, reducing sugars, saponins, steroids, tannins, and terpenes but it was absent in alkaloids (**Table 1**).

Antimicrobial activity: To determine the antimicrobial activity, the paper disc diffusion methods were used where the paper disc diameter is 8.0 mm and paper disc thickness is 0.5 mm. According to antimicrobial activity results, ethyl acetate extract showed medium activity on all six tested organisms. The methanol extract showed medium activity on *Bacillus subtilis*, *Staphylococcus aureus*, *Salmonella typhi*, and *Candida albicans* but low activity against *Bacillus pumilus* and *Escherichia coli*. In addition, n-hexane extracts of the crude sample displayed low activity in five tested organisms except *Staphylococcus aureus* (**Table 2** and **Figure 1**).

Antioxidant activity: The IC_{50} value is a parameter widely used to measure antioxidant activity. The radical scavenging activity was expressed in terms of IC_{50} (50.0% inhibition concentration). The lower the IC_{50} value, the higher the antioxidant activity. According to the result, the crude extract exhibited significant IC_{50} values of antioxidant properties which were compared with standard ascorbic acid. From the DPPH scavenging assay, the IC_{50} value of ascorbic acid was found to be 2.59 μg/mL and 36.28 μg/mL for ethyl acetate extract, 137.17 μg/mL for methanol extract, and 97.28 μg/mL for water extract. According to these results, the crude extract showed that ethyl acetate extract is higher than other extracts in antioxidant activities. Thus, crude methanol extract can be

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reextracted with ethyl acetate to isolate pure organic compounds. The selected medicinal plant is certainly a vital source for medicinal applications in a broad spectrum of health disorders. Further pharmacological evaluation as well as compound isolation and characterization of this herb would be used by methanol as a suitable solvent. Moreover, antioxidants including vitamin C, vitamin E, and certain minerals like selenium, may have a role in managing oxidative stress associated with diabetes [17]. Some studies suggest that antioxidants may help reduce complications related to diabetes, such as diabetic neuropathy [17]. The values of IC_{50} of standard ascorbic acid and crude extracts are shown in **Table 3** and the comparison of these values is shown in **Figure 2**.

Table 1: Phytochemical screening of *Elephantopus scaber* L.

Type of Compounds	Extract	Reagents	Observations	Remarks	References
Alkaloids	1.0% HCl	Dragendorff's reagent	No ppt	-	18, 19
		Wagner's reagent	No ppt	-	19, 20
		Mayer's reagent	No ppt	-	20-22
Carbohydrates	H_2O	1.0% α-naphthol, Conc: H_2SO_4	Red ring colour	+	19, 20
Flavonoids	EtOH	dil: HCl, Mg turning	Brown ppt	+	23, 24
Glycosides	H_2O	10% lead acetate	White ppt	+	25
Gum and Mucilage's	H_2O	Alcohols test	Brown ppt	+	20
Phenolic compounds	EtOH	10.0% $FeCl_3$ 1.0% $K_3Fe(CN)_6$	Bluish Black ppt	+	20, 21
Polyphenols	EtOH	1.0% $FeCl_3$	Greenish-blue colour solution	+	19, 21
Quinones	EtOH	Conc: HCl test	Green colour solution	+	26
Reducing sugars	H_2O	Benedict's solution	Green colour ppt	+	19, 20
Saponins	H_2O	Distilled water, shake	Froth	+	21, 27
Steroids	EtOH	Pet-ether, acetic anhydride, Conc: H_2SO_4	Green colour solution	+	20
Tannins	H_2O	10.0% Gelatin	Blue-green colour solution	+	28
Terpenes	EtOH	$CHCl_3$ Acetic anhydride, Conc: H_2SO_4	Red colour solution	+	24

Table 2: Antimicrobial activity of crude extracts of the plant of *Elephantopus scaber* L.

Solvent	Test organisms (mm)					
	Bacillus subtilis	***Bacillus pumilus***	***Staphylococcus aureus***	***Salmonella typhi***	***Candida albicans***	***Escherichia coli***
n-Hexane	14.00 (+)	14.25 (+)	15.25 (++)	-	14.30 (+)	14.00 (+)
EtOAc	16.35 (++)	16.30 (++)	17.40 (++)	17.00 (++)	17.25 (++)	17.45 (++)
MeOH	15.00 (++)	14.50 (+)	15.50 (++)	15.85 (++)	15.30 (++)	14.50 (+)

* Paper disc diameter=8 mm; Paper disc thickness=0.5 mm
10-14 mm = (+) Low; 15-19 mm = (++) Medium; 20 mm above = (+++) High

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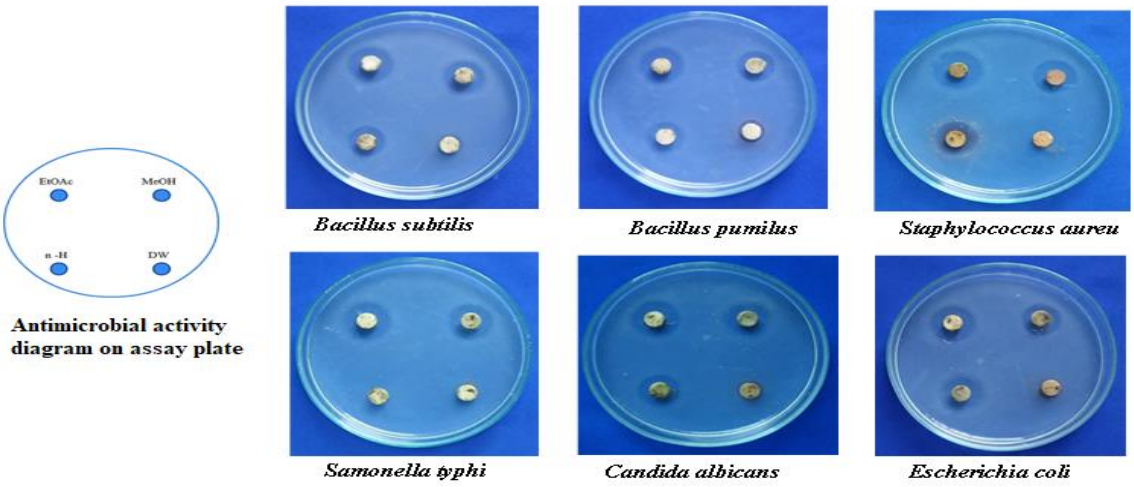

Figure 1: Antimicrobial activity of the various crude extracts for the plant of *Elephantopus scaber* L.

Table 3: IC_{50} values of standard ascorbic acid and crude extracts

Sample	Parameters	Results					IC_{50} (mg/mL)
Ascorbic Acid	Concentration	1	2	4	8	16	2.59
	% Inhibition	26.98	46.29	66.98	86.14	88.37	
Ethyl Acetate Extract	Concentration	160	80	40	20	10	36.28
	% Inhibition	84.25	61.75	52	41.25	35.5	
Methanol Extract	Concentration	400	200	100	50	25	137.17
	% Inhibition	74.26	66.58	40.84	25.74	20.54	
Water Extract	Concentration	400	200	100	50	25	97.28
	% Inhibition	87.75	70.25	53.75	40.75	32.25	

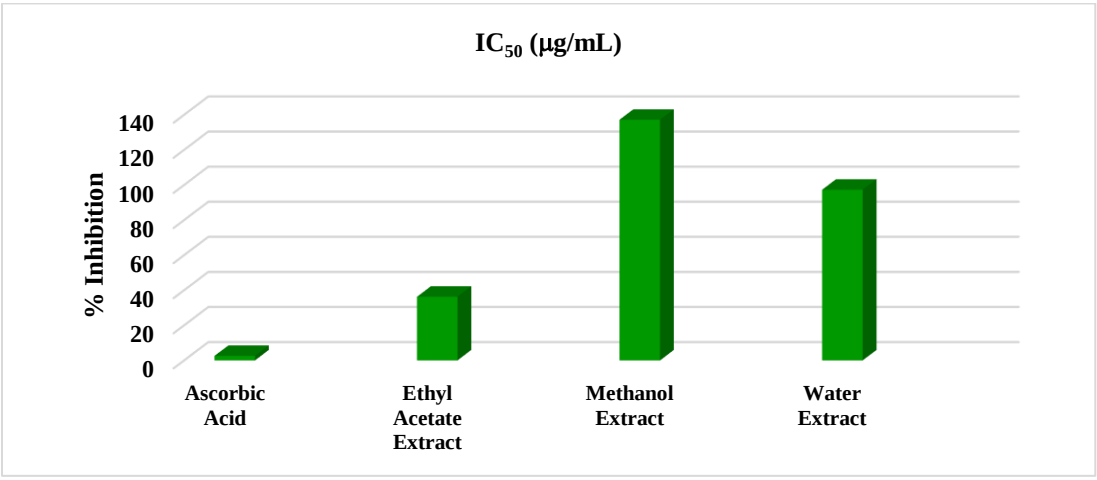

Figure 2: Inhibition of standard ascorbic acid and various extracts of the plant of *Elephantopus scaber* L.

Antidiabetic activity: Diabetes mellitus is a chronic endocrine metabolic disorder that affects the human body in terms of physical, psychological, and social health caused by defective insulin secretion, resistance to insulin action, or a combination of both. *In vivo*, antidiabetic activity was carried out by using experimental mice in this study. The blood glucose levels were measured over time under different conditions (**Table 4**). The effects of

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treatment (*Elephantopus Scaber L.*) were compared to a Negative Control, Positive Control, and Normal group, with glucose levels recorded at various time intervals (0 min, 45 min, 90 min, 135 min, 180 min, and 225 min). In the normal group, blood glucose levels fluctuate, peaking at 90 min (159 mg/dL) and then gradually decreasing. This can be considered the baseline physiological response to normal glucose metabolism without any intervention. The glucose levels stabilize around 121.4 mg/dL at 225 min, showing normal regulation of blood sugar levels (**Figure 3**). In the negative control, adrenaline administration leads to an initial spike in blood glucose levels at 45 min (164.8 mg/dL), likely due to adrenaline's role in promoting glycogenolysis and gluconeogenesis, leading to elevated blood sugar. Over time, glucose levels gradually decrease but remain higher than the initial level (126.8 mg/dL at 225 min), reflecting adrenaline's sustained effect on increasing blood glucose. In the positive control, glipizide, a standard hypoglycemic agent, shows an initial increase in glucose levels at 45 min (174.4 mg/dL), but the glucose levels decline steadily thereafter. By 180 min, glucose levels drop to 100 mg/dL, showing a significant reduction due to glipizide's mechanism of enhancing insulin secretion from the pancreas. This result confirms the effectiveness of glipizide in lowering blood glucose, reaching the lowest level among all groups by 180 min and remaining low (107.8 mg/dL) at 225 min. The extract of *Elephantopus scaberb* L. shows a noticeable hypoglycemic effect, with the blood glucose level increasing initially at 45 min (193.2 mg/dL), likely due to adrenaline. However, the glucose levels significantly decreased by 90 min (130.6 mg/dL) with a 32.0% reduction, continuing to decrease at 135 min (39.0% reduction) and 180 min (37.0% reduction). At 225 min, glucose levels still show a notable reduction (34.0% reduction) (**Figure 4**). The continuous reduction in blood glucose over time indicates that *Elephantopus scaberb* L. has a strong hypoglycemic potential, comparable to glipizide. By the comparison of groups, the Negative Control group remains slightly elevated throughout, possibly indicating a lack of glucose regulation (e.g., insulin deficiency or resistance). The Positive Control peaks sharply, but its glucose level also reduces gradually, showing the potential effect of a glucose-lowering agent. The *Elephantopus Scaber* L. group demonstrates a pattern where glucose levels rise and fall in a manner close to the Normal group, suggesting that this plant extract may help regulate blood sugar levels effectively. *Elephantopus scaberb* L. demonstrates a significant hypoglycemic effect, with reductions in blood glucose levels comparable to the standard drug, glipizide. The percentage reductions observed after 90 min (32.0%), 135 min (39.0%), 180 min (37.0%), and 225 min (34.0%) suggest that *Elephantopus scaberb* L. could be an effective natural remedy for managing hyperglycemia [29]. To conclude, *Elephantopus scaberb* L. presents itself as a promising candidate for further investigation in diabetes treatment due to its noticeable hypoglycemic effect. Further studies could explore its specific mechanisms of action, optimal dosages, and long-term effects on glucose regulation.

Table 4: Hypoglycaemic activity of *Elephantopus Scaber* L.

Groups	Dose	Blood glucose level mean (mg/dL) ± SD					
		0 min	45 min	90 min	135 min	180 min	225 min
Negative control (adrenaline)	0.2 mL/kg	110.0 ± 8.63	164.8 ± 21.28	152.6 ± 11.22	107.4 ± 10.33	114.4 ± 23.01	126.8 ± 32.45
Positive control (glipizide)	0.5 mg/kg	129.8 ± 12.28	174.4 ± 35.24	138.8 ± 21.25	113.2 ± 17.17	100.0 ± 18.36	107.8 ± 18.69
Normal	---	125.0 ± 17.28	145 ± 12.90	159.0 ± 6.28	128.6 ± 15.23	123 ± 10.02	121.4 ± 10.02
***Elephantopus Scaber* L.**	1.0 g/kg	133.6 ± 16.41	193.2 ± 66.22	130.6 ± 25.72	117.2 ± 19.51	121.2 ± 5.71	127.8 ± 18.4

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Acute toxicity: The toxicity is not only to determine the symptomatology consequence of ingestion of the drug but also to determine the nature and degree of toxicity produced by this drug. The acute oral toxicity test was carried out by using the limit test of OECD guidelines (425), in *vivo* mice model. The two oral doses (2000 mg/kg or 5000 mg/kg) were done (**Table 5**). If toxicity is observed at either dose, the dose is reduced for the next animal, and the process continues following the Up-and-Down method. The findings from this study indicate that *Elephantopus scaberb* L. does not induce mortality in the subjects at the tested concentrations of 2000 mg/kg and 5000 mg/kg, suggesting a favorable safety profile. Both doses yielded no adverse effects compared to a control group treated with 20.0% ethanol. These results imply that *Elephantopus scaberb* L. may be safe for further study, possibly in therapeutic or medicinal contexts. However, it is essential to consider the need for additional studies to explore the long-term effects, mechanisms of action, and potential benefits of *Elephantopus scaberb* L. Further studies should investigate varying doses, other endpoints (growth, behavior, or physiological changes), and different methods of administration to fully understand its pharmacological potential.

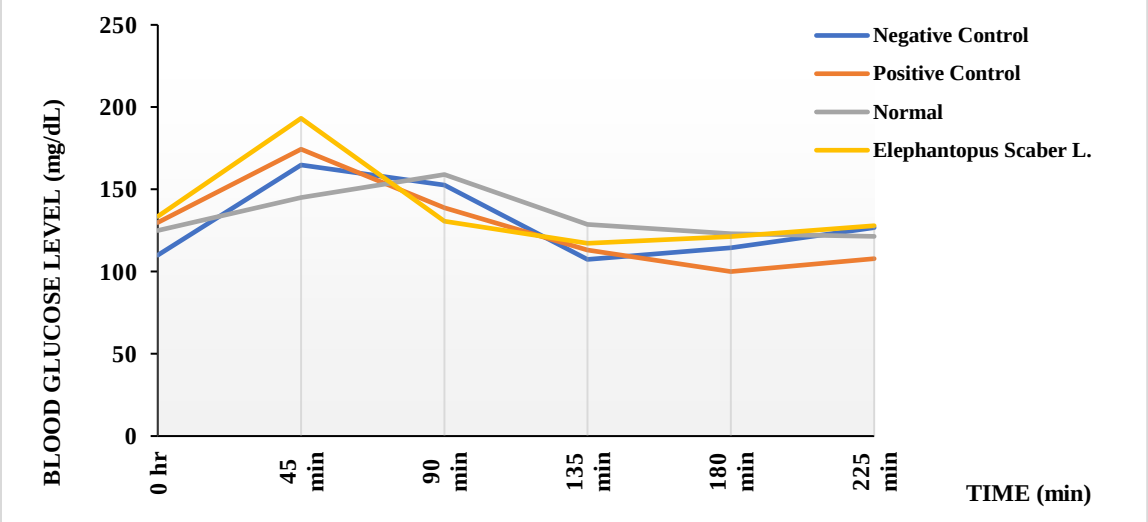

Figure 3: Effect of the extract of *Elephantopus scaberb* L. on the blood glucose of *Mus musculus*

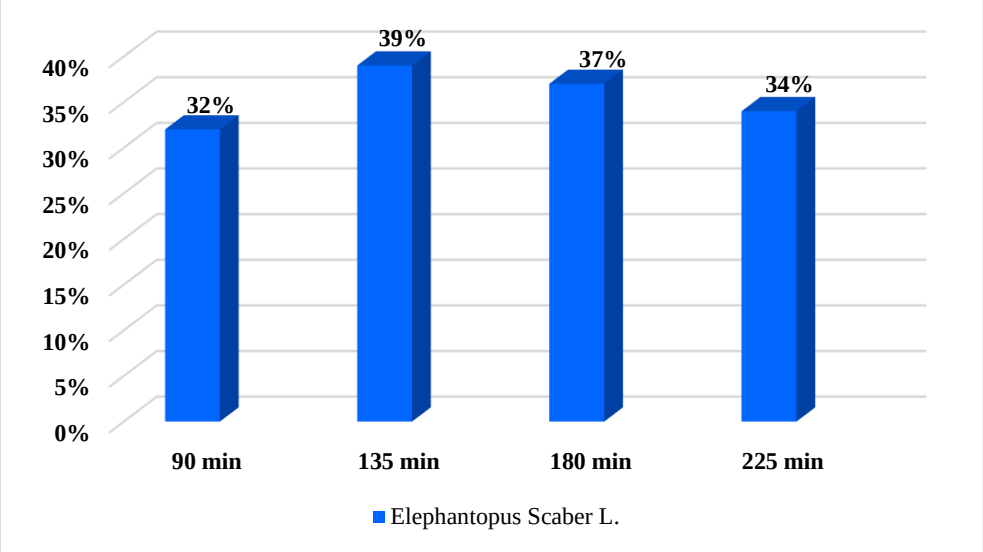

Figure 4: Reduction percentage of *Elephantopus Scaber* L. over time

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Table 5: Acute oral toxicity test by using a limit test of OECD guidelines (425), *in vivo* mice model

Date				Day									
Sample (mg/kg)		Total	Observation	1	2	3	4	5	6	7	8	9	10
***Elephantopus scaber* L.**	2000	5	Alive	5	5	5	5	5	5	5	5	5	5
			Dead	0	0	0	0	0	0	0	0	0	0
	5000	5	Alive	5	5	5	5	5	5	5	5	5	5
			Dead	0	0	0	0	0	0	0	0	0	0
Control	2.0 ml	5	Alive	5	5	5	5	5	5	5	5	5	5
		5	Dead	0	0	0	0	0	0	0	0	0	0

Conclusion: Elephantopus scaber L. shows antimicrobial, antioxidant, and antidiabetic activities. The significant effect of hypoglycemia could be an effective natural remedy for managing hyperglycemia. The findings from acute oral toxicity suggest a favorable safety profile.

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