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OCTOBER 29-30-31, 2025 / DEMIRE, ANTALYA, TÜRKIYE

PROCEEDINGS BOOK

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I[st] International Nature & Environment Conservation and Protected Areas Congress

"(INECPAC 2025)"

October 29-30-31, 2025
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(Abstracts & Full Texts)

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CONGRESS ID

Congress Title

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Date and Place

October 29-30-31, 2025, Demre, Antalya, Türkiye

Organizing

Demre Deputy Demre-Antalya, Türkiye

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PANEL

The Cultural Tourism Potential and Future of Demre District
(Demre İlçesinin Kültürel Turizm Potansiyeli ve Geleceği)

October 29, 2025 (Wednesday)

Antalya Time: 16:30 -18:30

Panel Venue
Demre Municipality Cultural Center Conference Hall
(Demre Belediyesi Kültür Merkezi Konferans Salonu)

Join Zoom Meeting
https://us02web.zoom.us/j/85971197642?pwd=N7LMLca
CiKtbbaZX4Lbj2cMSG4JRd0.1
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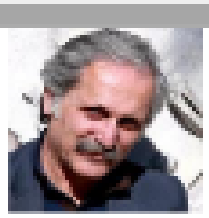

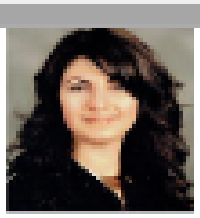

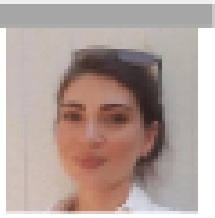

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Myra-Demre: Doğal ve Tarihsel Çevre ve Modern Yerleşimler	Demre Yöresinin Kültürel Mirasın Korunması ve Turizme Kazandırılması: Tekil Dönüşüm Örneğinden Fiziksel Yenileme Projelerine Genel Bir Bakış	"Yerel Miras için Yapay Zeka (AI) Sohbet Robotlarının İnce Ayarı: Aziz Nikolaos Örneği"	Demre'nin Sürdürülebilir Turizm Potansiyeli
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Akdeniz University, Faculty of Letters, Archaeology, Department of Classical Archaeology	Akdeniz University, Faculty of Architecture, Department of Interior Architecture	Akdeniz University, Demre Dr. Hasan Ünal Vocational School, Travel Tourism and Entertainment Services	Akdeniz University, Demre Dr. Hasan Ünal Vocational School, Travel Tourism and Entertainment Services

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(Online)

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MODERATOR: Busari M. B.

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International Nature & Environment Conservation and Protected Areas Congress
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Comparative *in Vitro* Antioxidant and Trypanocidal Activities of N-Hexane and Ethylacetate Extracts of *Azadirachta indica* on *Trypanosoma brucei brucei*

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Abstract

The parasitic disease known as trypanosomiasis remains a major health issue in Sub-Saharan Africa, affecting both humans and animals. In this study, phytochemical analysis, antioxidant analysis via ferric reducing antioxidant power, 2, 2-diphenyl-1-picrylhydrazyl and Inhibition of lipid peroxidation) as well as in vitro trypanocidal activities of n-hexane and ethylacetate extracts of Azadirachta indica (NAI and EAI) were carried out using standard methods. Blood obtained from infected donor mice was diluted with glucose phosphate buffered saline to contain 1x10⁷ parasites. Exactly 20 µL of the blood sample containing parasite was incubated with varying concentrations of 62.5 µg/mL, 125 µg/mL, 250 µg/mL and 500 µg/mL of NAI and EAI in separate microtiter plates in triplicate for 180 minutes. The mixture was observed under microscope for parasite motility at 10 minutes intervals. Quantity of the phytochemicals are present in the following order; phenol > saponins > tannins > alkaloids > flavonoids in both samples while EAI showed high antioxidant activities. Complete cessation of the parasite motility was observed for all test concentrations within 180 minutes of the study. The most active extract was EAI which caused complete cessation within 50 minutes in 500 µg/mL while that of NAI was within 90 minutes in 500 µg/mL when compared with berenil that occurred within 10 minutes at 500 µg/mL. These results showed that EAI has high trypanocidal and antioxidant activities when compared with NAI. Hence, EAI could explored further for the treatment of African Animal Trypanosomiasis.

Keywords: *Azadirachta indica*, trypanosomiasis, antioxidant, trypanocidal activities.

1. Introduction

Trypanosomiasis also known as sleeping sickness is a parasitic disease caused by protozoan parasites of the genus *Trypanosoma* (Ibrahim *et al.*, 2022). The disease is endemic in Sub-saharan Africa and is transmitted primarily by the bite of infected tsetse flies (*Glossina*

species). Human African Trypanosomiasis (HAT) manifests in two forms: the chronic form caused by *Trypanosoma brucei* gambiense, and the acute form caused by *Trypanosoma brucei* rhodesiense (Ibrahim *et al.*, 2022; Madaki *et al.*, 2022). This disease poses a significant public health challenge in many African countries, where poverty, limited healthcare infrastructure, and remote populations hinder effective treatment and control (Busari *et al.*, 2014; Kayode *et al.*, 2020).

In livestock, trypanosomiasis, also known as Animal African Trypanosomiasis (AAT), leads to significant economic losses due to reduced productivity, weight loss, and mortality in cattle, sheep, goats, and other animals. The disease burden in both humans and livestock not only affects individual health but also hampers agricultural productivity, contributing to food insecurity and poverty in affected regions (Maichomo *et al.*, 2021).

The current treatment options for trypanosomiasis are limited to a few drugs such as suramin, pentamidine, melarsoprol, and eflornithine, all of which have significant drawbacks. These drugs are associated with severe side effects, including toxicity, and the need for prolonged hospitalization for their administration. Moreover, drug resistance has emerged as a growing concern, further limiting the effectiveness of these treatments (Kayode *et al.*, 2020). As a result, there is an urgent need to explore alternative therapies that are safe, effective, and affordable for populations in endemic areas.

In recent years, researchers have turned their attention to natural products and medicinal plants as potential sources of new trypanocidal agents. Plants have been used in traditional medicine for centuries and are known to contain bioactive compounds that possess antimicrobial, antifungal, antiviral, and antiparasitic properties. One such plant is *Azadirachta indica*, commonly known as neem. Neem has been widely used in Ayurvedic and African traditional medicine to treat a variety of ailments, including parasitic infections (Wylie and Merrel, 2021).

Neem leaves contain a wide range of bioactive compounds, including limonoids, flavonoids, triterpenoids, and azadirachtin, which have shown promise in preclinical studies for their antimicrobial and antiparasitic activities (Hamzah *et al.*, 2023). Various parts of the neem tree, including the leaves, seeds, and bark, contain bioactive compounds that have been shown to possess antiparasitic, antimicrobial, and anti-inflammatory properties (Wasim *et al.*, 2023). Given the rich phytochemical profile of neem, its potential as a trypanocidal agent is an area of increasing interest (Wylie and Merrel, 2021). This study focuses on the *in vitro* trypanocidal activities of neem leaf extracts obtained using two solvents: n-hexane and ethylacetate, which are known for their efficacy in extracting different classes of bioactive compounds.

2. Materials and Methods

2.1 Materials

2.1.1 Plant material

Fresh *Azadirachta indica* leaves (neem) were obtained from Federal University of Technology, Gidan Kwano Campus, Minna, Niger State, Nigeria in July, 2023 and authenticated by Dr. O.A.Y. Daudu of the department of Biological Sciences, Federal University of Technology, Minna with voucher number FUT/PLB/MEL/001. The plant sample

was deposited in the herbarium of the department of Plant Biology, Federal University of Technology, Minna.

2.1.2 Chemicals and Reagents

The chemicals and reagents used includes: ethylacetate, n-hexane, dimethyl sulfoxide (DMSO), folin-ciocalteu's reagent, sodium carbonate, gallic acid, absolute methanol, aluminum chloride, sodium acetate, distilled water, folin-denis' reagent, standard tannic acid, petroleum ether, acetone-ethanol, ferrous sulphate, sulphuric acid, standard saponins, formaldehyde, acetate buffer, TPTZ solution, and ferric chloride solution, DPPH solution, linoleic acid, thiobarbituric acid, diminazene aceturate were all analytical standard.

2.1.3 Parasites

The parasite was sourced from stabilates preserved at the Nigerian Institute of Trypanosomiasis Research (NITR) in Kaduna, Kaduna State. It was maintained in the Animal House of the Department of Biochemistry, Federal University of Technology, Minna, through continuous passage into mice.

2.1.4 Site of Experiment

The experiment was conducted at the Centre of Genetic Engineering and Biocecology, Federal University of Technology, Minna, Bosso Campus. Minna is situated within the Southern Guinea Savanna region of Nigeria, at 9°33' N latitude and 9°37' E longitude.

2.2 Methods

2.2.1 Preparation of *Azadirachta indica* Leaves

The Azadirachta indica leaves were thoroughly washed with tap water to remove dirt and dried at 30 °C. Thereafter, the plant leaves were reduced to powder using the Silver Crest 2L Industrial 8500W electric blender, and the powder form was kept inside a tight covered plastic container.

2.2.3 Extraction of Bioactive Compounds

Extraction of *Azadirachta indica* leaf was done as reported by Busari *et al.* (2025). Briefly, exactly 230 grams of *Azadirachta indica* leaf powder was weighed into 1000 mL volumetric flask. Afterwards, 730 mL of each solvent was separately poured into flask and mixture was properly agitated and left for 72 hours with occasional vortexing each day. After the 72 hours, the solvent was evaporated using a water bath under reduced pressure before finally lyophilized with freeze drier. The freeze-dried sample was stored in tight covered glass containers and refrigerated at 10°C.

2.2.4 Phytochemical Screening (Quantitative Test)

2.2.4.1 Determination of phenolic content

Each of the n-hexane and ethylacetate extract of *A. indica* was dissolved in 0.01 g of 10 cm^3 distilled water, and 0.5 cm^3 of this solution was oxidized using 2.5 cm^3 of 10% Folin-ciocalteu reagent. The solution was neutralized with the addition of 2 cm^3 of 7.5% sodium carbonate. This mixture was then incubated at 45°C for a duration of 40 minutes. Following incubation, its absorbance was measured at 765 nm using a UV spectrophotometer. A calibration curve was generated using standard gallic acid (Singleton *et al.*, 1999). This process was also carried out for the ethylacetate plant extract.

2.2.4.2 Determination of flavonoid content

In a test tube, 1.5 cm^3 of absolute methanol was combined with 0.1 cm^3 of 10% aluminum chloride, 0.1 cm^3 of 1 M sodium acetate, and 2.8 cm^3 of distilled water. Then, 0.5 cm^3 of the n-hexane plant extract in n-hexane was added. The resulting mixture was incubated at room temperature for 30 minutes, after which its absorbance was measured at 415 nm using a UV spectrophotometer. The above process was also carried out for the ethylacetate extract. Standard quercetin was used to prepare the calibration curve. (Chang *et al.*, 2002).

2.2.4.3 Determination of tannin content

A 0.2 g of each sample of the plant extract was placed in a 50 cm^3 beaker, to which 20 cm^3 of 50% methanol was added. The beaker was then covered with parafilm and heated in an 80°C water bath for 1 hour. After heating, the mixture was thoroughly shaken to ensure uniform distribution and then filtered into a 100 cm^3 volumetric flask. To the filtered solution, 20 cm^3 of distilled water, 2.5 cm^3 of Folin-Denis reagent, and 10 cm^3 of sodium carbonate were added and mixed thoroughly. After standing at room temperature for 20 minutes, the solution developed a bluish-green color. The absorbance was taken at 760 nm using a UV-spectrophotometer. Standard tannic acid was used to prepare the calibration curve.

2.2.4.4 Determination of saponin content

A 0.5 g sample of the plant extract in n-hexane was weighed and dissolved in 20 cm^3 of 1N HCl, then heated in a water bath at 80°C for 4 hours. Once cooled, the mixture was filtered, and 50 cm^3 of petroleum ether was added. The ether layer was then separated and evaporated to dryness. The resulting dry residue was combined with 5 ml of a 1:1 acetone-ethanol mixture, 6 cm^3 of ferrous sulfate, and 2 cm^3 of concentrated sulfuric acid, and allowed to stand for 10 minutes. Absorbance was recorded at 490 nm. The same procedure was conducted for the ethylacetate extract, using standard saponins to establish the calibration curve (Oloyed, 2005).

2.2.4.5 Determination of alkaloid content

A 0.5 g sample of the n-hexane extract was weighed and dissolved in 5 cm^3 of a 1:1 mixture of 96% ethanol and 20% H_2SO_4, then filtered. One cm^3 of the filtrate was added to a test tube with 5 cm^3 of 60% H_2SO_4 and left to stand for 5 minutes. Following this, 5 cm^3 of 0.5% formaldehyde was added, and the mixture was kept at room temperature for 3 hours. Absorbance was measured at 565 nm. The same procedure was applied to the ethyl acetate extract, using the extinction coefficient of vincristine (E296, EtOH = 15136 M^{-1} cm^{-1}) as a reference for alkaloids (Oloyed, 2005).

2.2.5 Antioxidants Activities Assay

2.2.5.1 Ferric Reducing Antioxidant Assay

The FRAP reagent was prepared by mixing acetate buffer, TPTZ solution, and ferric chloride solution. The *Acadiranthe lediea* n-hexane extract was then added to the FRAP reagent, incubated for 30 minutes, and its absorbance was measured at 593 nm using a spectrometer. The antioxidant capacity was assessed by comparing the change in absorbance to that of the standard. The same procedure was followed for the ethylacetate extract of *Acadiranthe lediea*.

2.2.5.2 The 2,2-Diphenyl-1-picrylhydrazyl radical scavenging

A DPPH solution was prepared in methanol, to which the n-hexane extract was added and thoroughly mixed. The mixture was then incubated in the dark for 30 minutes, followed by absorbance measurement at 517 nm. This process was also applied to the ethyl acetate extract. The percentage of radical scavenging activity was calculated in comparison to a control.

2.2.5.3 The Inhibition of Lipid Peroxidation

Linoleic acid was homogenized mixed with each of the extract. The mixture was incubated at 37 °C for 30 minutes. Thiobarbituric acid (TBA) reagent was added to the mixture, each mixture was then heated to form a colored MDA-TBA complex (Malondialdehyde – Thiobarbituric). The percentage of lipid peroxidation inhibition was calculated compared to a control.

2.2.6 Trypanocidal Study

2.2.6.1 Test Organism

The *Trypanosoma brucei brucei* was sourced from stabilates maintained at the Nigerian Institute of Trypanosomiasis Research (NITR) in Kaduna, Nigeria, and kept in the Animal House through repeated passaging in mice until required. Passages were conducted when parasitemia levels reached between 16 and 32 parasites per field, generally occurring 3 to 5 days post-infection. For each passage, 1×10^5 parasites in 0.1 to 0.2 mL of blood were injected intraperitoneally into mice that had been acclimated to laboratory conditions for two weeks. (Abedo *et al*., 2015).

2.2.6.2 *In vitro* Trypanocidal Activity

The *in vitro* trypanocidal activity assessment was conducted in quadruplicate using 96-well microtiter plates (Tewabe *et al*., 2014). A 0.5 g sample of the n-hexane plant extract was dissolved in 10 ml of distilled water to create a 0.05 g/ml stock solution (equivalent to 50,000 µg/ml). From this stock, solutions of 500 µg/ml, 250 µg/ml, 125 µg/ml, and 62.5 µg/ml were prepared, resulting in four different concentrations. The same procedure was applied to the ethylacetate plant extract.

A 20 µl blood sample containing approximately 20–25 parasites per microscopic field was mixed with 5 µl of each test concentration, specifically 500 µg/ml, 250 µg/ml, 125 µg/ml, and 62.5 µg/ml, respectively. To verify that any effects observed were specifically from the extract, two control setups were established. The first control used Berenil (Diminazene aceturate, a reference drug) as the positive control, while the second control consisted of blood mixed with glucose-phosphate buffered saline as the negative control. Berenil was prepared to match the concentrations of the test solutions.

Each test mixture was incubated for 5 minutes in sealed microtiter plates at 37°C. Then, 2 µl of each test mixture was placed on separate microscope slides, covered with 7×22 mm cover slips, and the parasites were observed every 10 minutes for a total of 60 minutes, monitoring for any cessation or reduction in motility, using a 400 X Objective lens (Tewabe *et al*, 2014).

3. Findings and Discussions

3.1 Quantitative phytochemical screening

The table below shows the phytochemical analysis of *Azadirachta indica* (neem) extracts. The analysis revealed significant differences in the concentrations of bioactive compounds between the n-hexane and ethylacetate extracts. The ethylacetate extract exhibited higher levels of phenols (134.49 ± 0.7 mg/g), tannins (42.81 ± 0.5 mg/g), saponins (101.85 ± 0.8 mg/g), and alkaloids (5.85 ± 0.2 mg/g) compared to the n-hexane extract. These compounds are known for their potent antioxidant, antiparasitic, and membrane-disrupting properties, which may explain the higher trypanocidal activity observed in the ethylacetate extract. While the n-hexane extract had higher flavonoid content (3.33 ± 0.4 mg/g), the overall lower concentrations of key phytochemicals suggest that the ethylacetate extract contains more bioactive compounds responsible for its stronger antitrypanosomal effects.

Table 1. Quantitative phytochemical composition of N-hexane and ethylacetate extract of *Azadirachta indica*

	Concentration (mg/100g)				
Sample	**Phenols**	**Flavonoids**	**Tannins**	**Saponins**	**Alkaloids**
NAI	114.73±0.6	3.33±0.4	30.43±0.5	87.01±0.2	4.05±0.8
EAI	134.49±0.7	1.39±0.5	42.81±0.5	101.85±0.8	5.85±0.2

NAI – n-hexane extract of *Azadirachta indica*, EAI – ethylacetate extract of *Azadirachta indica*

3.2 Antioxidants Activities of N-hexane and Ethylacetate extracts of *Azadirachta indica*

3.2.1 Ferric Reducing Antioxidant Power of N-hexane and Ethylacetate extracts of *Azadirachta indica*

The antioxidant capacities of the n-hexane and ethylacetate extracts of *Azadirachta indica* were evaluated using the FRAP assay at various concentrations (500 µg/ml, 250 µg/ml, 125 µg/ml, and 62.5 µg/ml), as presented in the table below. The results indicate that the ethylacetate extract exhibited stronger ferric reducing antioxidant power (FRAP) at all tested concentrations compared to the n-hexane extract. At the highest concentration (500 µg/ml), the ethylacetate extract showed the highest FRAP value (73.08±0.1), while the n-hexane extract displayed a lower value (65.45±0.3). As the concentration decreased, the FRAP values for both extracts reduced accordingly, with the ethylacetate extract maintaining higher activity even at the lowest concentration (62.5 µg/ml), where it recorded a FRAP value of 19.02±0.2 compared to 15.93±0.2 for the n-hexane extract. This suggests that the ethylacetate extract possesses superior antioxidant capacity, which may contribute to its higher trypanocidal activity.

Table 2. Ferric Reducing Antioxidant Power of N-hexane and Ethylacetate extracts of *Azadirachta indica*

Sample	**Concentrations**			
	500µg/ml	**250µg/ml**	**125µg/ml**	**62.5µg/ml**
NAI	65.45±0.3	51.20±0.3	31.82±0.2	15.93±0.2
EAI	73.08±0.1	50.21±0.3	44.93±0.2	19.02±0.2

NAI – n-hexane extract of *Azadirachta indica*, EAI – ethylacetate extract of *Azadirachta indica*

3.2.2 The 2, 2-Diphenyl-1-picrylhydrazyl of N-hexane and Ethylacetate extracts of *Azadirachta indica*

The DPPH (2,2-Diphenyl-1-picrylhydrazyl) assay was performed to assess the free radical scavenging capabilities of the n-hexane and ethylacetate extracts of *Azadirachta indica* at various concentrations (500 µg/ml, 250 µg/ml, 125 µg/ml, and 62.5 µg/ml). The ethylacetate extract showed stronger antioxidant activity across all concentrations compared to the n-hexane extract. At the highest concentration (500 µg/ml), the ethylacetate extract exhibited the highest DPPH radical scavenging activity (62.81±0.2), while the n-hexane extract had a lower activity (54.54±0.2). As the concentration decreased, the free radical scavenging ability of both extracts diminished. At the lowest concentration (62.5 µg/ml), the ethylacetate extract still showed higher activity (9.77±0.2) compared to the n-hexane extract (3.02±0.2). These results indicate that the ethylacetate extract possesses better antioxidant activity, which may contribute to its stronger trypanocidal efficacy.

Table 3. The 2,2-Diphenyl-1-picrylhydrazyl of N-hexane and Ethylacetate extracts of *Azadirachta indica*

Sample	Concentration			
	500 µg/ml	250 µg/ml	125 µg/ml	62.5 µg/ml
NAI	54.54±0.2	41.21±0.3	22.96±0.1	3.02±0.2
EAI	62.81±0.2	47.66±0.2	29.71±0.3	9.77±0.2

NAI – n-hexane extract of *Azadirachta indica*, EAI – ethylacetate extract of *Azadirachta indica*

3.2.3 The Inhibition of Lipid Peroxidation of N-hexane and Ethylacetate extracts of *Azadirachta indica*

The ILP (Inhibition of Lipid Peroxidation) assay was performed to assess the ability of n-hexane and ethylacetate extracts of *Azadirachta indica* to prevent lipid peroxidation at different concentrations (500 µg/ml, 250 µg/ml, 125 µg/ml, and 62.5 µg/ml). The ethylacetate extract exhibited stronger inhibitory effects on lipid peroxidation across all concentrations compared to the n-hexane extract. At 500 µg/ml, the ethylacetate extract demonstrated the highest inhibition (50.33±0.2), while the n-hexane extract showed lower inhibition (42.31±0.7). As the concentrations decreased, the inhibition of lipid peroxidation declined for both extracts. Even at the lowest concentration of 62.5 µg/ml, the ethylacetate extract maintained a higher inhibition value (3.12±0.2) compared to the n-hexane extract (1.54±0.1). These results indicate that the ethylacetate extract is more effective in preventing lipid peroxidation, likely due to its higher content of bioactive compounds, contributing to its overall antioxidant and trypanocidal activities.

Table 4. The Inhibition of Lipid Peroxidation of N-hexane and Ethylacetate extracts of *Azadirachta indica*

Sample	Concentration			
	500µg/ml	250µg/ml	125µg/ml	62.5µg/ml
NAI	42.31±0.7	32.91±0.2	19.32±0.2	1.54±0.1
EAI	50.33±0.2	37.29±0.2	26.22±0.2	3.12±0.2

NAI – n-hexane extract of *Azadirachta indica*, EAI – ethylacetate extract of *Azadirachta indica*

3.3 Effect of Different Concentrations of N-hexane and Ethylacetate Extracts of *Azadirachta indica* on Motility of *T. Brucei brucei*

The Table 5 presents the effects of different concentrations of n-hexane and ethylacetate extracts of *Azadirachta indica* on the motility of *Trypanosoma brucei brucei*. At 500 µg/ml, the n-hexane extract ceased the parasite's motility after 90 minutes, while the ethylacetate extract was more effective, ceasing motility in 50 minutes. As the concentrations decreased, the time taken for motility cessation increased for both extracts but less effective than diminazene Aceturate which took 10 minutes cessation at the same concentration.

Table 5. Effect of N-hexane and Ethylacetate extracts of *Azadirachta indica* on Motility of *T. brucei brucei*

Test Substance	Time (Minutes) after which motility ceased with different concentrations of extracts Parasite motility (µg/ml)			
	500 µg/ml	250 µg/ml	125 µg/ml	62.5 µg/ml
AIN	90.40 ± 8.30	130.20 ± 10.60	180.40 ± 12.10	240.60 ± 15.50
AIE	50.40 ± 5.10	90.30 ± 6.10	150.40 ± 8.10	190.70 ± 10.10
Diminazene Aceturate	10.20 ± 1.15	10.50 ± 1.20	10.60 ± 1.30	30.80 ± 2.90

Value was expressed as the mean of triplicate ± standard deviation

NAI = n-hexane extract of *Azadirachta indica*, EAI = ethylacetate extract of *Azadirachta indica*

The findings showed higher antioxidant activates in EAI might be as a result of content of phenols, tannins and flavonnids. These compounds contain hydroxyl groups that can serve as reactive oxygen species quenchers (Husari *et al*., 2021). The same effect that was revealed by EAI on higher exhibition of trypanocidal effects might be due to higher phytochemicals such as flavonoids, tannins, phenolics, and alkaloids, which have been reported to possess strong antiprotozoal and antiparasitic properties (Rahman *et al*., 2020). These phytochemicals are known for their ability to induce oxidative stress in parasites, disrupt cellular processes, and inhibit enzyme activities essential for parasite survival. The higher trypanocidal activity observed in the ethylacetate extract may be attributed to these compounds ability to interfere with the metabolism and reproduction of the parasite, ultimately leading to its death (Patel *et al*., 2022). In fact, the site and number of hydroxyl groups on polyphenols are thought to be related to their relative toxicity to microorganisms, with evidence that increased hydroxylation results in increased toxicity (Eid *et al*., 2017). This explains why polyphenols present in neem were more effective in parasite reduction. The ability of the neem plant extract to lower the levels of parasitaemia can therefore be attributed to the toxic activity of polyphenols present in it. Indeed, polyphenols have been shown to have anti-trypanosomal activity (Eid *et al*., 2017).

Although, ability of NAI to show less trypanocidal activities than the ethylacetate extract suggests that less polar solvent could still extract potent compounds that can still play a role in combating *T. brucei brucei* existence. However, the superior performance of the ethylacetate extract suggests that the polar solvent could extract compounds from *A. indica* that may have a stronger affinity for intracellular targets within the parasite. This finding is consistent with

previous research indicating that *A. indica* extracts contain a variety of bioactive compounds with potential antitrypanosomal properties. This study reinforces the idea that *Azadirachta indica* could be a promising natural source of trypanocidal agents, with the ethyl acetate extract showing particularly strong activity in this regard (Rahman *et al.*, 2020).

4.Conclusion and Recommendations

Hence, *in vivo* antioxidants, trypanocidal and toxicities studies of ethylacetate extract should be carried out to affirm the antioxidant and trypanocidal effects as well as safety of the extract. The active compound(s) should also be isolated in order to obtain the trypanocidal drug candidate as well as to unravel the mechanism through which the isolated compound(s) exhibit their trypanocidal activities.

Conflict of Interest

Finally, all authors contributed equally to the article to the success of this article and no conflict of interest between the Authors.

Ethical Approaval

The article complies with national and international research and publication ethics. Although, Ethics Committee approval was not required for the study because it was an *in vitro* studies

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