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Evaluation of the Cytotoxicity of *Terminalia avicennioides* plant extract, using the Brine Shrimp Lethality Test (BSLT) on *Drosophila melanogaster* Flies

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Abstract

The aim of the current study is to identify and quantify the bioactive compounds present in *Terminalia avicennioides* extracts and assess their biological activity using a combination of phytochemical screening, characterization techniques (FT-IR) and cytotoxicity evaluation. The phytochemical analysis revealed the presence of several bioactive compounds, including alkaloids, flavonoids, tannins, saponins, steroids, and phenols, which are known for their medicinal properties. Spectroscopic analysis of the extract carried out using Fourier Transform Infrared Spectroscopy (FT-IR) indicated the presence of O-H stretching vibration (3224.46 cm^{-1}), an sp^3 C-H stretching vibration of Alkanes (2923.56 cm^{-1}), C=O stretching vibration of carbonyl group (1709.26 cm^{-1}), and C-O stretching vibration of an ester. (1175.84 cm^{-1}). The cytotoxicity tests of the extract were evaluated at different concentrations of the extract (50 mg/ml, 100 mg/ml, 250 mg/ml, 500 mg/ml) using the Brine Shrimp Lethality Test (BSLT) and tested on *Drosophila melanogaster*, a decreased mortality rate was observed when the concentration of the extract was increased, the negative geotactic response in *Drosophila* decreased with increase in the concentration of the extract, indicating a dose-dependent reduction in motor coordination or neurological function, while a non-linear relationship was observed for the enclosure emergence. The results demonstrated significant bioactivity that suggests potential therapeutic applications. The findings of the study validate the traditional use of *Terminalia avicennioides* in herbal medicine and highlight its potential as a source of new natural products for pharmaceutical development.

Keywords: **Brine Shrimp Lethality Test (BSLT), Cytotoxicity, *Drosophila melanogaster*, *Terminalia avicennioides***

Introduction

Terminalia avicennioides is locally referred to as "Baushe" among the Hausa, 'Kpace" in Nupe, "Kpayi" in Gwari, "Idi" among the Yoruba. It grows as shrub or small tree with short bole to 10 m high, sometimes bushy and branching from the base. It is a tree with yellowish brown, hard and durable wood. Several bioactive hydrolysable tannin compounds including ellagic acid, punicalagin, flavogallonic acid and terchebulin have been isolated from this plant, and none of them have been shown to have antimycobacterial activity at low concentrations. The root bark of *T. avicennioides* contains anthraquinone, saponins, steroids, tannins and terpenes [1].

The insufficient data on the cytotoxicity activity of the *Terminalia avicennioides* extract, poses a major problem in its evaluation for medical applications and raising concerns about the potential health risks. The chemical composition remains poorly characterized, limiting the understanding of its pharmacological properties. However, this project seeks to explore the medicinal and therapeutic potential of *Terminalia avicennioides* extract, with due consideration of the cytotoxicity level when employed for therapeutic drugs; so, safety concerns and dosage considerations are pivotal aspects that needs clarification. [2].

Materials and Methods

Sample Preparation

The fresh plant material of *Terminalia avicennioides* was obtained from Kaduna metropolis, Kaduna State, Nigeria. The plant sample was authenticated at the Herbarium Section of the Department of Biological Sciences, Kaduna State University, Nigeria, and was given a voucher number (KASU/BSH/999).

Extraction of Plant Material

The method of extraction used for this experiment was maceration. The plant sample was air-dried at room temperature at around 25°C and pulverized using a mortar and pestle to obtain a fine powder, which was sieved through a sieving material with a mesh size of 0.8 mm in diameter. The pulverized sample was weighed (1:5 w/v) and soaked in n-hexane for 72 hours with constant shaking. The resulting extract was then filtered, and the filtrate was subjected to evaporation using a water bath [3]. The extraction was carried out on three different parts of the plant (*Terminalia avicennioides*) separately which are the leaf, root and stem bark. This was adopted in order to screen the respective phytochemicals present in the different parts of the sample extracts.

Phytochemical Screening

Phytochemical examinations were conducted on the different parts of the plant extract (leaf, root and stem bark) using standard procedures to ascertain their constituents. The qualitative analysis of the crude extracts was performed according to the methods described by Maiyama *et al* [4] to identify the presence of various classes of phytochemicals present in the extracts as follows:

Detection of phenolic

Ferric chloride test: the presence of phenol was tested by adding 10 ml of the extract with few drops

of ferric chloride solution (20%). A bluish black colour indicated a positive test.

Test for flavonoids

Sulphuric acid test: The presence of flavonoid was tested using sulphuric acid test of which 0.5 cm^3 of the extract was treated with few drops of concentrated H_2SO_4. The formation of orange colour indicated the presence of flavonoid**.**

Sodium hydroxide test: The extract was dissolved in 1 cm^3 of water to which two drops of 10% Sodium hydroxide are added. A yellow colouration indicated the presence of flavonoids.

Ferric chloride test: Three drops of Ferric chloride solution were added to the solution of the crude extract. A green colour indicated the presence of flavonoids. The addition of dilute HCl made the solution colourless.

Detection of terpenoids

The presence of terpenoids was tested using Salkowskis test, 1 ml of the extract was mixed with 2 ml chloroform and 3 ml of concentrated H_2SO_4 was added to form a layer. A layer of reddish brown colour appeared in the interface to indicate a positive test.

Detection of saponins

The presence of saponin was detected by mixing 0.5 mg of extract with 5 ml of distilled water in a test tube and was shaken vigorously. Formation of a stable foam, indicates the presence of saponins.

Detection of steroids

To detect the presence of saponin, 2 ml of the extract with 2 ml of chloroform and concentrated H_2SO_4 was added. It produces a red colour in the lower chloroform layer.

Test for Alkaloids

Dragendorff's Test: The extract (0.2g) is dissolved in 2 cm^3 of 1% aqueous hydrochloric acid with continuous stirring in a water bath. The mixture is then filtered, and 3 drops of Dragendorff's reagent are added. A rose-red precipitate indicates the presence of alkaloids.

Mayer's Test: To 2 cm^3 of the acidic solution of the extract in a test tube, 3 drops of Mayer's reagent are added. A cream precipitate indicates the presence of alkaloids.

Detection of Tannins

To detect the presence of tannins, 0.5 ml of the extract with 1 ml of distilled water were weighed and 3 drops of 10% ferric chloride was added. A blue or green black colour indicated a positive test.

Detection of cardiac glycoside

To test the presence of cardiac glycoside, a solution of glacial acetic acid (4.0) was mixed with 1 drop of 2.0% $FeCl_3$, then 10 ml aqueous plant extract and 1 ml of concentrated H_2SO_4.

A brown ring between the layers showed a positive test.

Characterization

Characterization of the plant extract was carried out using Fourier Transform Infrared Spectroscopy (FT-IR) in accordance with the method of spectral analyses reported by Ogbiko *et al.* [5]

***FT-IR Spectroscopy*:** Fourier Transform Infrared Spectroscopy (FT-IR) was carried out at Ahmadu Bello University, Zaria, Kaduna State, Nigeria. This was carried out in order to investigate the functional groups present in the plant extract obtained.

Cytotoxicity Test

Cytotoxicity test was carried out using the plant extract obtained from *Terminalia avicennioides on Drosophila melanogaster* (fruit flies) to determine how toxic the extract is. This is crucial for assessing the safety of chemicals, drugs, or natural extracts, especially when considering them for therapeutic purposes.

Drosophila melanogaster Stock and Culture:

Wild-type *Drosophila melanogaster* (Harwich strain) fruit flies were obtained from the National Species Stock Centre (Bowling Green, OH, USA). The flies were bred and nurtured on cornmeal medium containing brewer's yeast (1% w/v), agar-agar (1% w/v), and nipagin (preservative, 0.08% v/w) at a constant temperature of 24 ± 2 °C and 70% humidity under a 12 hour dark/light cycle in the Drosophila Laboratory, Department of Biochemistry, Faculty of Physical Sciences, Kaduna State University, Kaduna State, Nigeria.

Toxicity Studies (LC_{50}) and Incapacitation Action Test of the Alkaloid Extract on Drosophila melanogaster;

Mortality Study (LC_{50}): The toxicity study was evaluated as described by [6] with slight modifications. The fruit flies were divided into nine different groups, each receiving varying concentrations of the alkaloid extract ranging from low to high concentration (50 mg, 100 mg, 250 mg, 500 mg), which were incorporated into 10 g of the flies' diet. These were then transferred into empty sterilized treatment vials, with group 1 containing only 10 g of the diet as a normal control (diet only). The experiment was conducted in triplicates, with each vial containing twenty fruit flies of both sexes. The flies are placed in the vials containing the diet and alkaloid extract. On the third day, the flies were transferred into newly prepared diets with the same alkaloid extract concentrations. The mortality rate of the flies was recorded every 24 hours for seven days.

Determination of Negative Geotaxis: A negative geotaxis assay was employed to determine the locomotor performance of the flies, as previously reported by Omotayo *et al.*[7] The flies from both the control (containing diet only) and the treated groups were transferred into an empty vial. The flies are gently tapped to the bottom of the vial, and the number of flies that climbed above the 6 cm

mark within 6 seconds was recorded. The results were presented as a percentage of the total number of flies in each group.

Reproductive Ability (Eclosion Assay): Twenty fruit flies of both sexes aged 1-4 days were transferred into treatment vials containing the diet and different concentrations of the alkaloid extract (50 mg/10 g, 100 mg/10 g, 250 mg/10 g, 500 mg/10 g). The flies were allowed to feed on the diet for three days before being transferred to new vials with fresh diets. The old vials were kept until day 10, when the eclosion assay was conducted. The eclosion assay was performed to evaluate the emergence of new offspring by counting the number of pupal cases in each vial, including the normal control vials. The number of pupal cases is recorded.

Results and Discussion

Table 1 shows that phenolic chemicals, terpenoids, and tannins were strongly present in *Terminalia avicennioides* leaf extract. The presence of tannins and phenolic compounds suggests that the extract may have strong anti-inflammatory and antioxidant qualities. Although more research is required, terpenoids' high concentrations raise the possibility of their antibacterial or anticancer properties.

The leaf extract's lack of flavonoids and saponins indicates that these substances are either absent or just present in trace amounts. Since flavonoids have anti-inflammatory and antioxidant qualities, their absence may affect the leaf extract's overall medicinal value. Although not as powerful, the presence of cardiac glycosides and alkaloids suggests some degree of bioactivity. While cardiac glycosides may have an impact on cardiovascular health, alkaloids are frequently linked to pharmacological effects like analgesic and antimalarial qualities. The chemical profile of the leaf as a whole points to a variety of medicinal potential that merits more research. For the aerial portion of *Terminalia avicennioides*, the results obtained here are consistent with those obtained by [8].

Table 2 indicates that phenolic chemicals, flavonoids, saponins, and tannins are highly present in *Terminalia avicennioides* root extract. This suggests that the root contains a lot of antioxidant and maybe antibacterial compounds. The root has a unique chemical profile, as seen by the lack of terpenoids and steroids compared to results from other plant sections. Unlike other plant parts, the root may not have major cardiovascular effects, as indicated by the absence of cardiac glycosides. The high presence of some phytochemicals in the root extract could be attributed to the functions of plant morphology, which means that the underground organs have in storage most of the compounds and nutrients [9].

Phytochemical Screening Results for Leaf, Root, and Stem Bark

Table 1: Result of the Phytochemical Screening of the Leaf Extract

Phytochemical	Detection
Phenolic	++
Flavonoids	-
Terpenoids	++
Saponins	-
Steroids	-
Tannins	++
Alkaloids	+
Cardiac Glycosides	+

Present (+); high presence (++); undetected (-)

Table 2: Result of the Phytochemical Screening of the Root Extract

Phytochemical	Detection
Phenolic	++
Flavonoids	++
Terpenoids	-
Saponins	++
Steroids	-
Tannins	++
Alkaloids	+
Cardiac Glycosides	-

Present (+); high presence (++); undetected (-)

As demonstrated in Table 3 above, *Terminalia avicennioides* stem bark extract has substantial concentrations of tannins, steroids, saponins, and phenolic compounds. The stem bark may have strong antibacterial, anti-inflammatory, and antioxidant qualities, according to this extensive profile. Additionally, the presence of steroids suggests possible analgesic and anti-inflammatory effects. Additional putative pharmacological

activity, such as potential antibacterial and cardiovascular effects, are indicated by the moderate detection of cardiac glycosides and terpenoids. While cardiac glycosides may provide therapeutic benefits for heart-related illnesses, terpenoids may add to the stem bark's overall medicinal usefulness. The absence of flavonoids contrasts with the high levels of other phytochemicals, indicating that while the stem bark has a diverse chemical profile, it lacks some of the antioxidant benefits associated with flavonoids. However, the stem bark's total phytochemical composition indicates that it has a variety of possible medical uses. For the aerial portion of *Terminalia avicennioides*, the results obtained here are consistent with those obtained by [8]

Table 3: Result of the Phytochemical Screening of the Stem Bark Extract

Phytochemical	Detection
Phenolic	++
Flavonoids	-
Terpenoids	+
Saponins	++
Steroids	++
Tannins	++
Alkaloids	+
Cardiac Glycosides	+

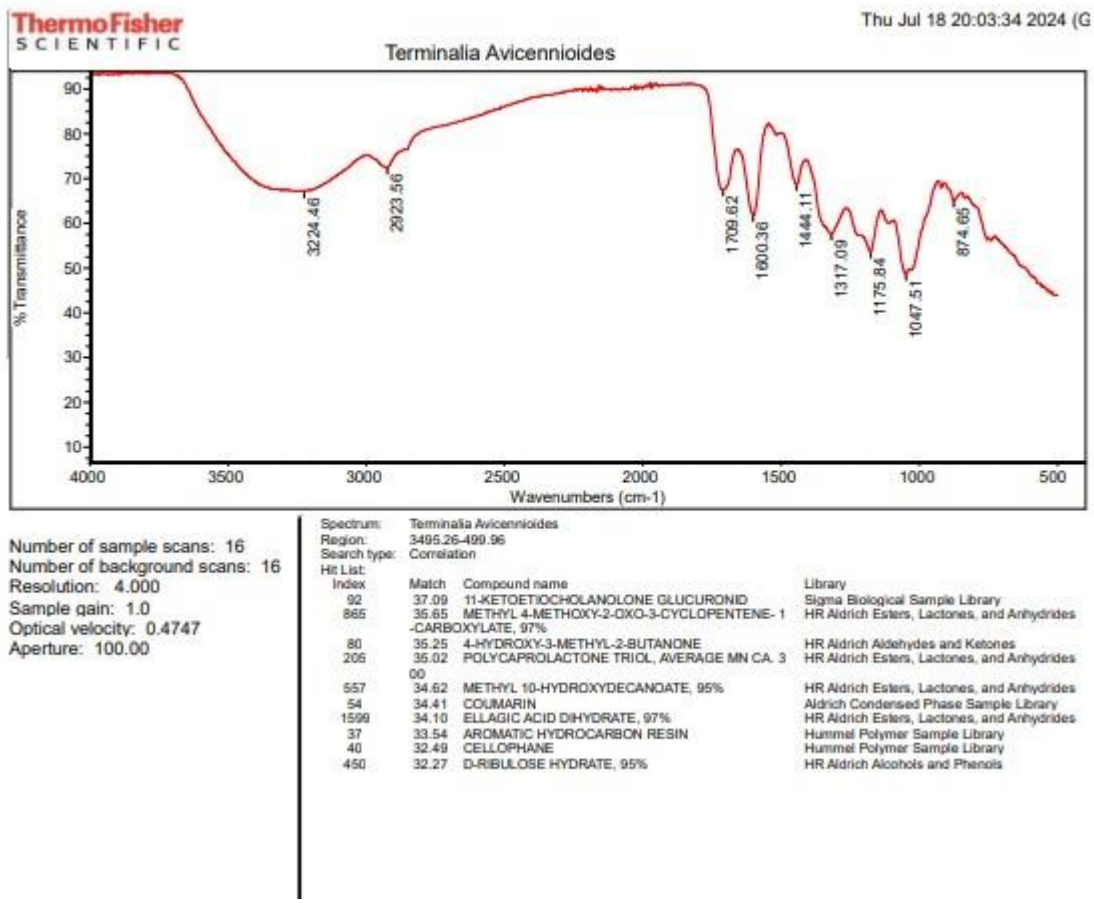
Present (+); high presence (++); undetected (-)

In this study, we examined the extract's chemical profile and correlated these findings with specific compounds identified in the spectroscopic library, drawing connections between the phytochemical constituents and their known biological activities.

Correlation with 11-Ketoetiocholanolone Glucuronide

One of the compounds identified in the spectroscopic analysis was 11-ketoetiocholanolone glucuronide, a metabolite commonly associated with anti-inflammatory properties. Alkaloids present in *Terminalia avicennioides* are known to exhibit similar biological activities, suggesting that

the presence of alkaloids in the extract could be responsible for the identification of this compound. Alkaloids are renowned for their ability to modulate inflammatory pathways, making the correlation with 11-ketoetiocholanolone glucuronide particularly relevant to the plant's traditional use in managing inflammation**.** [10]



Methyl 4-Methoxy-2-Oxo-3-Cyclopentene-1-Carboxylate

The identification of methyl 4-methoxy-2-oxo-3-cyclopentene-1-carboxylate in the spectroscopic library points to the presence of esterified compounds in the *Terminalia avicennioides* extract. Esters are often derived from flavonoids and phenolic acids, both of which were detected in the phytochemical analysis. These compounds contribute to the antioxidant properties of the plant, neutralizing free radicals and preventing oxidative damage. The presence of this ester in the extract reinforces the potential antioxidant activity linked to the flavonoid content of *Terminalia avicennioides*. [10]

4-Hydroxy-3-Methyl-2-Butanone

4-Hydroxy-3-methyl-2-butanone, a compound identified through the spectroscopic analysis, is commonly associated with ketones found in natural extracts. The presence of this compound may be linked to the saponins detected in the phytochemical screening of *Terminalia*

avicennioides. Saponins are known for their emulsifying properties and their role in enhancing the bioavailability of other phytochemicals. The identification of this ketone suggests a structural relationship with the saponins, which may contribute to the overall pharmacokinetics of the extract. [10]

Polycaprolactone Triol

Polycaprolactone triol, identified in the analysis, is a polymer-related compound often associated with biodegradable materials. While not a typical plant metabolite, its presence in the analysis may be indicative of the glycosidic structures found in the plant extract. Glycosides, which were detected in the phytochemical analysis, are known for their role in plant defense and their ability to form complexes with other bioactive compounds. The correlation with polycaprolactone triol may suggest a structural similarity between these glycosides and synthetic polymers.

Methyl 10-Hydroxydecanoate

Methyl 10-hydroxydecanoate is another ester identified in the spectroscopic analysis, likely reflecting the esterified fatty acids present in the *Terminalia avicennioides* extract. Fatty acids, though not the primary focus of the phytochemical analysis, are important components of the plant's lipid profile. The presence of this ester suggests that the extract contains fatty acid derivatives, which could contribute to the overall bioactivity, particularly in terms of antimicrobial and anti-inflammatory effects. [10]

Coumarin

The detection of coumarin in the spectroscopic analysis aligns with the tannins identified in the phytochemical screening. Coumarins are a class of compounds known for their anticoagulant and anti-inflammatory properties, often derived from tannin-rich sources. Tannins in *Terminalia avicennioides* are known to possess astringent properties, which are useful in treating wounds and infections. The presence of coumarin reinforces the potential therapeutic uses of the extract in managing blood-related disorders and inflammation.

Ellagic Acid Dihydrate

Ellagic acid dihydrate, identified in the spectroscopic analysis, is a potent antioxidant found in many tannin-rich plants. The detection of this compound correlates with the high tannin content observed in the *Terminalia avicennioides* extract. Ellagic acid is known for its ability to scavenge free radicals and protect cells from oxidative stress, supporting the use of this plant in traditional medicine for its antioxidant properties. The presence of ellagic acid further highlights the potential of *Terminalia avicennioides* as a natural source of antioxidants. [10]

Aromatic Hydrocarbon Resin

The identification of aromatic hydrocarbon resin in the analysis may be linked to the complex polyphenolic compounds present in the extract. Polyphenols, which include tannins and flavonoids,

contribute to the plant's antioxidant and antimicrobial activities. The presence of this resin suggests a structural similarity to the polyphenolic compounds, which could explain some of the plant's observed biological effects, such as its ability to inhibit microbial growth and protect against oxidative damage.

D-Ribulose Hydrate

D-ribulose hydrate, a sugar-related compound identified in the spectroscopic analysis, could be associated with the glycosides detected in the phytochemical screening. Sugars and glycosides are integral to the plant's metabolic processes, serving as building blocks for more complex molecules. The presence of D-ribulose hydrate suggests that the glycosidic content of *Terminalia avicennioides* may contribute to its overall bioactivity, particularly in terms of energy metabolism and cellular protection. [10]

Cytotoxicity Results

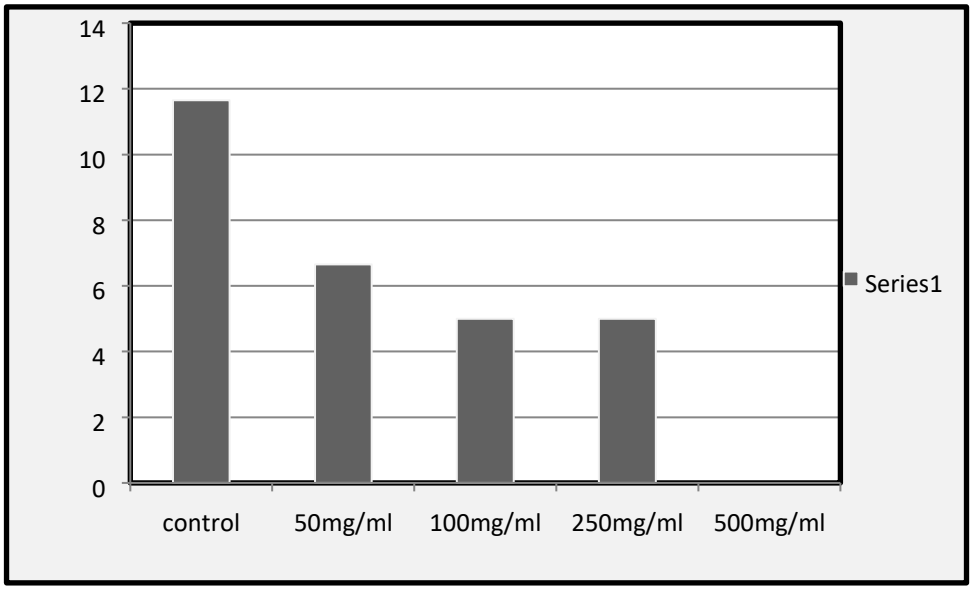


Fig. 1: Effect of Concentration on Mortality (% Mortality)

Fig. 1 displays the relationship between different concentrations of *Terminalia avicennioides* stem bark extract and the percentage of mortality observed in *Drosophila melanogaster*. The concentrations tested were: control (no extract), 50 mg/ml, 100 mg/ml, 250 mg/ml, 500 mg/ml, and the following were observed:

Control Group (with no extract) represents the baseline mortality rate of the fruit flies under normal conditions without any exposure to the extract. And the Mortality rate observed here was 11.65%. At 50

mg/ml concentration (mortality 6.65%) a noticeable decrease in mortality compared to the control group was observed, suggesting a potential protective effect at this concentration. At 100 mg/ml and 250 mg/ml concentration (mortality:5%), a further reduction in mortality was recorded, indicating the extract continues to have a non-toxic or beneficial effect. A zero mortality rate was recorded at 500 mg/ml concentration, indicating the highest protective effect or complete lack of toxicity at this concentration.

With this, it is safe to say that as the concentration of *Terminalia avicennioides* extract increases, the percentage of mortality in *Drosophila melanogaster* decreases. This inverse relationship suggests that the extract is not cytotoxic to the fruit flies within the tested concentration range. The extract may possess antioxidant properties that protect the fruit flies from natural mortality factors. The result obtained here is in contrast with that obtained by [11] when the toxic effect of *Drimia maritima* extract on *Drosophila melanogaster* was investigated. Their result showed a positive correlation with the extract concentration used.

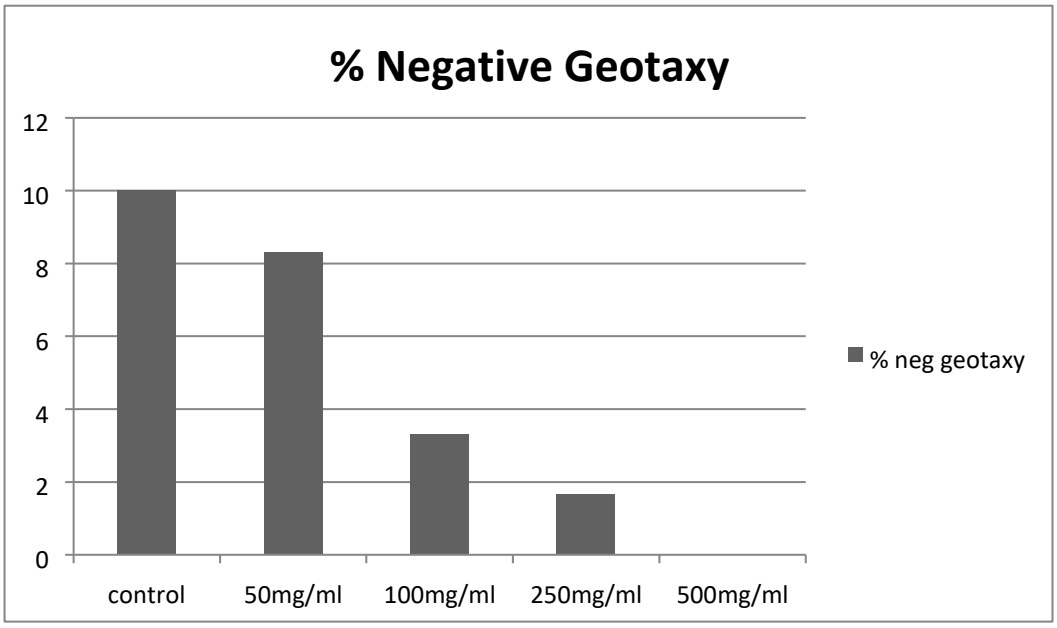


Fig. 2: Effect of concentration on Negative Geotaxy

Negative geotaxis is the natural tendency of fruit flies to move against gravity. The y-axis displays the percentage of flies showing this behaviour while the x-axis represents different concentrations of the extract. As observed from fig. 2, the control group had the highest percentage (10%) of negative geotaxis, indicating a strong geotactic response without exposure to the extract. While at 50mg/ml concentration, a slight decrease to 8.3% geotaxis suggests a mild impairment in mobility at this

concentration. At 100mg/ml, the percentage dropped significantly to 3.3%, showing a more pronounced reduction in negative geotaxis. Similarly, at 250mg/ml concentration, the response was further reduced to 1.65%, indicating a substantial impairment in geotactic behaviour. A negative geotaxis behaviour was completely absent (0%) at 500mg/ml concentration, which implies a severe impairment or loss of motor function.

As the concentration of *Terminalia avicennoids* extract increases, the negative geotactic response in *Drosophila* decreases, indicating a dose-dependent reduction in motor coordination or neurological function. This suggests that higher concentrations of the extract have a significant impact on the flies' ability to perform this reflexive behaviour potentially due to neurotoxicity or other biological effects. Similar findings were recorded in previous research when D. maritima extract showed a possible presence of neurotoxic property when treated on *Drosophila melanogaster* at higher concentrations [11].

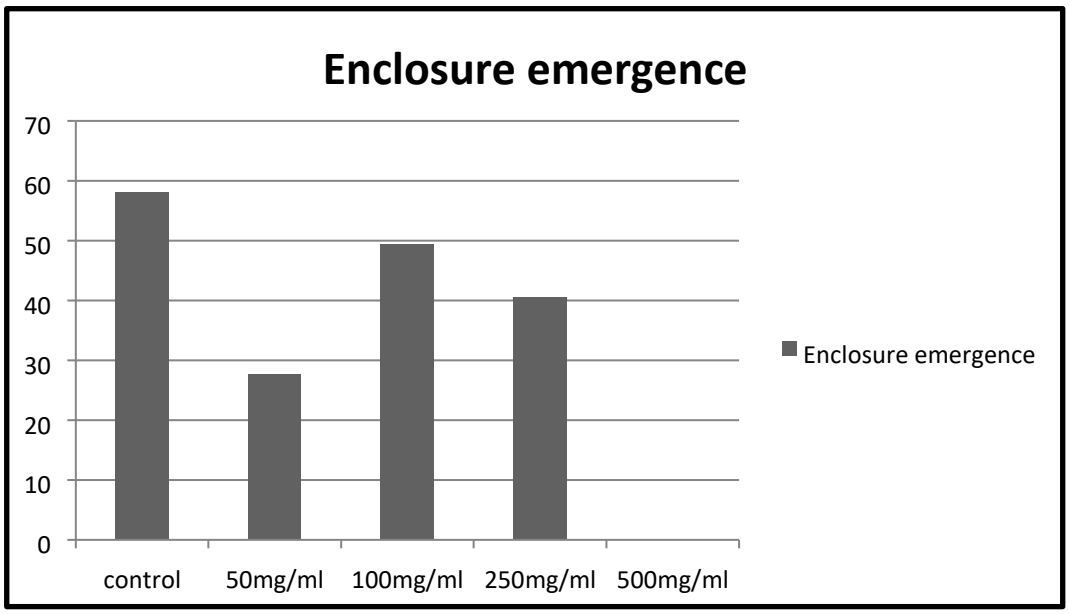


Fig. 3: Effect of concentration on enclosure emergence in *Drosophila melanogaster*

The enclosure emergence refers to the ability of Drosophila larvae to successfully develop into adult flies. The y-axis shows the percentage of enclosure emergence, and the x-axis represents different concentrations of the extract. As observed from the graph (Fig. 3): Control group had the highest enclosure emergence (58%), reflecting a healthy developmental process without the extract. At 50 mg/ml concentration, there was a significant drop in enclosure emergence to 27.6%, indicating developmental inhibition at this concentration. But at 100mg/m concentration, the emergence increased to 49.3%, suggesting a recovery of developmental capacity

at this concentration. At 250mg/ml concentration, the percentage dropped to 40.6%, showing a reduction in successful development compared to 100mg/ml. As recorded in both percentage mortality rate and percentage negative geotaxy, the 500 mg/ml concentration showed a zero-emergence percentage (0%), indicating a complete block in the development of larvae into adult flies at this concentration.

The data shows that the effect of *Terminalia avicennoids* extract on enclosure emergence in *Drosophila* is non-linear. While low to moderate concentrations (50mg/ml and 250mg/ml) result in significant developmental inhibition, a higher concentration (100mg/ml) appears to allow for greater emergence, though it still inhibits development compared to the control. At the highest concentration (500mg/ml), no flies emerge, suggesting that this concentration may be lethal or severely disruptive to larval development. This pattern indicates a complex interaction between the extract and the developmental process in *Drosophila*.

Conclusion

Known for their therapeutic qualities, the phytochemical screening identified phenols, flavonoids, terpenoids, saponins, steroids, tannins, and alkaloids. The plant's potential as a useful source of natural treatments is highlighted by the effective identification and characterization of these compounds utilizing spectroscopic technique (FT-IR). Additionally, the plant's bioactivity was shown in the cytotoxicity tests on *Drosophila melanogaster*, suggesting that it could be used to create novel treatments for a number of illnesses.

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