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PHYTOCHEMICAL SCREENING OF FOUR SAHARAN PLANTS FROM SOUTHWEST ALGERIA: INSECTICIDAL ACTIVITY OF SAHARAN PLANT EXTRACTS AGAINST THE STORED GRAIN PEST *TRIBOLIUMCASTANIUM* (HERBST, 1797) (COLEOPTERA: TENEBRIONIDAE)

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Abstract:

Studies on phytochemicals are now playing out the initial stage in developing effective medications. In addition to being valuable for biological activity, weeds are the richest source of drugs. In this study, a qualitative phytochemical investigationwas carried into four different Saharan plants (***Datura stramonium L., Hyoscyamus murices L., Pergularia tomentosa L., and Calotropis procera A.***), belonging to the families of Solanaceae and Asclepiadaceae. Saponins, flavonoids, steroids, unsaturated sterols, terpenes, and alkaloids were among the phytochemicals found in the plant extracts, according to their phytochemical screening. However, some plant extracts were devoid of cardenolides, tannins, and unsaturated sterols. In a second experiment, the insecticidal properties of several leaf extracts from four different plants were tested against adult stages of the stored grain pest ***Triboliumcastanium,*** using the ***no-choice*** test technique.The contact application for ***T. castanium***during five days of treatment with butanol extracts of ***P. tomentosa*** resulted in ***100%*** mortality, with a lethal time (TL_{50}) of ***577.62minutes***.The findings show that ***P. tomentosa*** possesses insecticidal properties against ***T. castanium,*** making it a viable substitute for synthetic insecticides in grain protection.

Keywords: phytochemical screening, ***Triboliumcastanium***, insecticidal activity, ***Calotropis procera, Pergularia tomentosa, Datura staramonium, Hyoscyamus muticus*.**

1. INTRODUCTION

Algeria's diverse flora, a rich and abundant source of medicinal compounds, resultsfrom its huge size and variable climate. A significant reservoir of many plants that have not yet been thoroughly studied is found in the Sahara region.

Phytochemicals are bioactive substances derived from plants. We refer to them as secondary metabolites because the plant that produces them might not need them. Every component of the plant, including the bark, leaves, stem, roots, flowers, fruits, and seeds, is naturally synthesised with them. Any portion of the plant's body might include active ingredients. (**Inas*et al*., 2017).**Understanding the chemical components of plants is important since this knowledge may be used to create intricate chemical compounds. The primary focus of plant studies is the exploration of new secondary metabolites that exhibit many pharmacological characteristics (**Praveen *et al*., 2014).**The medicinal and bioactive importance of plants is due to the presence of some special bio-substances like alkaloids, glucosides, resin, volatiles oils, gum and tannins, etc **(Soni et al., 2011).** Consequently, the investigation of chemical components would improve the identification of different biological and biochemical activities of plants **(Bachir Raho Ghalem *et al*., 2017).**Among these bioactive components, alkaloids, tannins, flavonoids, and phenolic substances are the most significant ones **(Doss, 2009;Nandagoapalan*et al.,* 2016).**

In thisresearch, four plants were subject to characterization and phytochemical studies, say: *Datura stramonium* L., *Hyoscyamus muticus* L., *Pergularia tomentosa* L., and *Calotropis procera*(Ayton) W.T. Aiton ***(Figure 1).***

(a). Pergulariatomentosa. (b). Hyoscyamus muticus. (c) Datura stramonium.(d). Calotropis procera.

Figure 1: The selected and collected plantsfrom their natural home area**(*Bourmita, 2013)*.**

C. procera, an Asclepiadaceae plant ***(Figure 1)***, is an important Ayurvedic herb with notable therapeutic properties **(Dirir *et al*., 2017).** It is a well-known plant and has been traditionally usedfor *diarrhea, stomatic, diabetes, and skin disease*.In traditional medicine, different components of these plants have been used to cure a variety of conditions, including leprosy, ulcers, tumours, piles, and illnesses affecting the spleen, liver, and abdomen. This plant's extracts with water, ethanol, acetone, and other organic solvents have insecticidal, larvicidal, antibacterial, and antiparasitic properties**(Wadhwani *et al.*,2021;Suman, et *al*.,2024).**

The plant is used to cure leprosy, leukoderma, liver, and abdomen. It has also been claimed tohave possible anthelmintic, antibacterial, anticancer, anticoagulant, analgesic, anti-inflammatory, purgative, and antipyretic qualities **(Morsy *et al.,* 2016).** Latex present in the plant contains abortifacient, spasmogenic, and antidysentery, anti-syphilitic, antirheumatic, as well as antifungal activities. In addition, it may be used to treat skin disorders and bronchial asthma **(Bashyal., *etal*., 2017).**

D. stramonium commonly known as Jimson weed or ***Datura(Figure 1),*** belongs to the family of Solanaceae **(Iqbal et al., 2017).**In various cultures, the natural plant compounds have also been employed as narcotics and local anaesthetics **(Ananth, 2013).**

Most plant's chemical components are present throughout the whole plant, which has several historical uses. Alkaloids, scopolamine, tannins, flavonoids, saponins, and phenols are the main psychoactive components of all plants in the genus Datura. These compounds have antimicrobial, anti-asthmatic, anti-cancer, anti-proliferative, free radical scavenging, and hypoglycaemic properties **(Shobha, 2014).**

H. muticus L., commonly known as ***Btima(Figure 1)***or Egyptian henbane from family of Solanaceae **(Mohd*et al*., 2017)**is an abundant family of phytochemicals with medicinal qualities, including tropane alkaloids **(Elsharkawy et al., 2018).** The main alkaloids found in ***H. muticus*** are scopolamine, nor-scopolamine, hygrine, nor-hygrine, tropine, homo-tropine, pseudo-tropine, and hyoscyamine. According to reports, the leaf and root extract contains other phytoconstituents such as steroids, isofucosterol, and muticin **(Mohd et al., 2017)**. Anti-asthmatic, anticholinergic, anaesthetic, narcotic, and spasmolytic qualities are among the medical applications **(Bosila et al., 2016, Walla M. Abed Elmaksood et al., 2016; Mohd et al., 2017).**

Asclepiadeceae is the family that includes ***P. tomentosa***, or milkweed **(*Figure 1*) (Alghanem et al., 2017)**. The Saharan area is home to this perennial plant mostly **(Shinkafi, 2014).** It is frequently employed in traditional medical practices for a variety of reasons. Numerous substances are present in it, such as anthraquinones, flavonoids, saponin glycosides, alkaloids, cardiac glycosides, and tannins **(Al-Mekhlafi et al., 2017)**. ***P. tomentosa*** was employed for treating diarrhoea, and the leaves' sap was used topically and believed to be a stand-alone treatment for head disorders **(Lahmar et al., 2017)**. Skin problems, including tinea capitis, have been treated with milk extract from ***P. tomentosa*** leaves **(Al-Mekhlafi et al., 2017)**.

Using synthetic pesticides, insect control for stored items can now be effectively achieved. However, the widespread use of pesticides has resulted in a number of adverse impacts, including pesticide resistance, toxicity to people and non-target creatures, pesticide residue, and environmental risks **(Chun-Xue and Arthur, 2017)**.

The use of plant extracts and herbal essential oils for pest control has drawn the attention of several researchers in recent years. **(Mahmoudi Manesh et al., 2022)**; researchers are paying particular attention to bioinsecticides as a potential method of pest management. It is previously known that plant extracts and phytochemicals work as insecticides, growth inhibitors, repellents, and antifeedants. The use of botanical pesticides has prompted research into the effectiveness of different botanical extracts as insecticides **(Melanie et al., 2022)**.

Plant extracts and essential oil constituents are the primary sources of antifeedant plant materials. These resources are studied in a variety of forms, including the essential oil (***EO***) and active fractions of crude extract, as well as in further processing screening processes to isolate pure active chemicals **(Melanie et al., 2022).**

This study aims to determine how well four Saharan plant leaf extracts screened for phytochemicals and proved insecticidal against adult stages of the stored grain insect ***Triboliumcastanium*** (Herbst).

2. MATERIALS AND METHODOLOGY

2.1. Collection of Sample Plants

The parts of four plants screened in this work were collected from their natural area in***October 2014 and April 2015(Table 1),*** from ***Taghit-Bechar*** (***southwest of Algeria***). Different parts of plants include leaves, stems, whole fruits, and whole flowers of four plants ***D.stramonium, H.muticus, P. tomentosa, and C. procera.***

A sample for the herbarium was collected, recognised, and verified with botanical authentication.

The vegetable material was shaded and subsequently separated, then dried under shade at room temperature until all plant components were thoroughly desiccated. The plant components were

dried, then chopped into little pieces and kept in glass bottles until they were ready to be used **(Nia et al., 2015).**

Table 1: Details of the samples plants species considered for preliminary phytochemical extracts and insecticidal activity.

Name and family of the studied species	**Vernacular name**	**Area of collection**	**Date of collection**	**Condition of plant material**	**Used parts**
*Calotropis procera***Asclepiadaceae CA04/02**	Kranka	Taghit ***(Bechar, Algeria)***	13/10/2014	Fresh	Leaves Stems Fruits
*Hyoscyamus muticus***Solanaceae CA00/43**	Btima	Taghit &Kenadssa ***(Bechar, Algeria)***	17/10/2014	Fresh	Leaves Stems Whole flowers
*Datura stramonium***Solanaceae CA00/50**	Alhabala	Taghit ***(Bechar,Algeria)***	12/04/2015	Fresh	Leaves Stems Whole fruits
Pergulariatomentosa **Asclepiadaceae CA00/44**	Alrelgha	Taghit ***(Bechar,Algeria)***	26/10/2014	Fresh	Leaves Stems

2.2. Phytochemical screening

To identify a variety of distinct phytochemicals, material for phytochemical screening in different chemical tests was extracted and prepared, say: saponins, flavonoids, steroids, unsaturated sterols, terpenes, alkaloids unsaturated sterols, cardenolides, and tannins based on the protocols available in the literature**(Karande, 2016).**

A separate extraction was performed using ***50***milligrams of powdered plant material, ***95%*** ethanol, methanol, chloroform, acetone, and distilled water. by allowing it to macerate for a whole night at room temperature, filtering it using ***Whatman's*** filter paper ***No.1***, and using a water bath to concentrate the solutions. Prior to examination, the extracts were tagged and stored in various containers within polythene bags. Various chemical tests were performed on the extracts in accordance with different methodologies for the identification of distinct phytochemicals. The phytochemical screening of the extracts was done using the techniques of **Sofowora (1982), Harborne J. B. (1984), Trease and Evans (2002), Gini et al. (2013), and Mehta Sonam et al. (2017).**

2.3. Used Insects

The bioinsecticide activity tests on the red flour beetle ***T. castanium***was employed to investigateinsecticidal properties that have been obtained from natural plant extracts. They were carried out in an isolated room with obscure conditions at the experimental laboratory research of the ***University TAHRI Mohamed of Bechar*** to have a large adult population.The breeding was performed on the flour in transparent glass containers and mesh to allow good ventilation. The containers were filled to ***¾*** with flour then around ***30*** insects were introduced.The containers were then closed to prevent the disappearance of the insects. These containers were placed in an oven or a warm place (fastest growth at ***30-34 °C*** and relative humidity of **75-80 %**) **(*May and June 2017*).**

2.4. Toxicity test (*Bioassaymethods*)

The activity of the extracts was tested on ***T. Castanium*** using a "no-choice" test with a 10% concentration of each extract. Ten individuals of the adults of ***T. Castanium*** were placed on Petri dishes (***10 cm in diameter***) to check ***0% to 100%*** mortalities. Mortality counts were taken after 5 days of treatment.

By applying the plant extract directly to insects, its insecticidal action was established. Ten mature insects were contained in a Petri dish, and ***1 µl*** was systematically pushed into the thorax of each fly in order to achieve a concentration by dilution. The controls received the same treatment but were simply given water. Four repetitions of each concentration and control were made. The mortality rate was calculated five days after treatment. When insects showed no signs of movement, either in their legs or antennae, they were declared dead. We counted the percentage of insect mortality using Abbott's formula adjustment method for natural mortality in untreated controls **(Brik and Frah, 2021; Rojht et al., 2009).**

2.5. Statistical analysis

With approaches reported in research of ***Elango G., (2012),*** the average mortality rates of termites were examined using probit analysis to calculate TL_{50} using the **Schneiderformula (Abbott, 1925 and Bourmita et al., 2013).** The information concerning mortality was subject to probit analysis, as described by **Finney(1971),** to get the TL_{50}values. The **Schneiderformula** was used for this study, and the results were considered statistically significant when the Pearson's correlation coefficient was less than **0.05(Bourmita et al., 2013).**

$$MC = \left(\frac{M2 - M1}{100 - M1}\right) \times 100$$

Whereas:

M_C =Corrected mortality (%)

M_2 =Mortality rate in the group (%)

M_1= Mortality rate in the non-treated control group (%)

Graph Pad Prism version 5.01 software was used to generate and analyse the standard deviations for chi-square, t-significance, correlation, and ***ANOVA***. Results with $P<0.05$ proved to be statistically significant.

3. RESULTS AND DISCUSSION

The four plant extracts were subject to a phytochemical evaluation, which indicated the presence of bioactive substances such as tannins, cardenolides, saponins, flavonoids, steroids, unsaturated sterols, and terpenes, as well as alkaloids (***Table2***).

Table 2: Phytochemical evaluation results of the four studied plants.

Species	Used parts	Saponins	Tannins	Unsaturated sterols, and terpenes	Alkaloids	Steroids	Unsaturated Sterols	Cardenolides	Flavonoids
C. procera Asclepiadaceae **CA04/02**	Leaves	+++	++	+++	++	++	+	++	++
	Stems	+	+	+	+++	+	-	+++	++
	Fruits (pulps)	++	+	+	+	++	+	+	+
D. stramonium Solanaceae **CA00/50**	Leaves	+	++	++	+	+	-	++	++
	Stems	+	-	++	+	+	+	+	++
	Whole fruits	+	-	+	++	+	+	-	+
H. muticus Solanaceae **CA00/43**	Leaves	+	+	++	++	++	-	+	+
	Stems	++	++	+	+	+	+	+++	++
	Whole flowers	+	+	+	++	+	-	+	+
P. tomentosa Asclepiadaceae **CA00/44**	Leaves	+	+++	++	++	+++	+	+++	++
	Stems	+++	-	+++	+	+	+	++	+

Keys:• Absent (-) • Weak presence (+) • Average presence (++) • Strong presence (+++)

The procedure previously described was used to perform a phytochemical evaluation of crude plant extract from various parts of the four tested plants. The extract was then analysed for phytocompounds such as saponins, flavonoids, steroids, unsaturated sterols, terpenes, alkaloids, unsaturated sterols, cardenolides, and tannins. The preliminary analysis revealed that all of the tested plants contained saponins, flavonoids, steroids, unsaturated sterols, and terpenes, with the exception of some sections where unsaturated sterols, cardenolides, and tannins were absent ***(Table 2)***.

The findings of the phytochemical analysis (flavonoids test) conducted on the four plants are reported and shown in the table below ***(Table 3)***. The presence of flavonoids, free flavonoids, heterosidic flavonoids, and glycoside flavonoids was found in the phytochemical screening findings of the four plants (flavonoids test) using part extracts of ***C. procera, Datura stramonium, Hyoscyamus muticus, and Pergularia tomentosa(Table 3).***

The extracts under evaluation differ in the concentrations of the different types of secondary metabolites. Water is the component with the highest concentration, followed by butanol, ethyl acetate, dichloromethane, and hexane.This species may have some therapeutic promise based on the existence of these components.

Table 3: Phytochemical screening results of the four plants ***(flavonoids test)***

Plants	Used parts	Flavonoids	Free Flavonoids	Heterosidic Flavonoids	Glucoside Flavonoids
Calotropis procera Asclepiadaceae ***CA04/02***	Leaves	++	+	-	+
	Stems	++	+	-	+
	Fruits (pulps)	+	-	-	+
Datura stramonium Solanaceae ***CA00/50***	Leaves	++	+	-	+
	Stems	++	-	-	+
	Whole fruits	+	-	-	+
Hyoscyamus muticus Solanaceae ***CA00/43***	Leaves	+	-	-	+
	Stems	++	-	+	+
	Whole flowers	+	-	-	+
Pergulariatomentosa Asclepiadaceae ***CA00/44***	Leaves	++	-	-	++
	Stems	+	-	-	+

Keys: • Absent (-) • Weak presence (+) • Average presence (++) • Strong presence (+++)

There are harmful effects of various concentrations on mature ***T. castanium.*** Several statistical findings, including the ***lethal time 50*** (**TL_{50}**) (min), the 95% confidence limit, and the chi-square values, were obtained from the probit analysis of the mortality rate and are shown in ***Table 4*** after a period of exposure. The highest level of residual toxicity was seen in adult ***T. castanium***, corresponding to the rise in extract efficacy with exposure duration ***(Table 4)***. In the control group, there was no mortality.

Table 4: Impact of different plant extract activities on the ***50%*** mortality ***lethal time*** of the ***Tribolium castanium*** population.

Plants used	*Extract type*	*Regression*	*Regression coefficient (R^2)*	*Lethal time 50 (TL_{50}) (min)*
Pergulariatomentosa CA00/44	Hexane	y = 1.945x - 0.766	R^2 = 0.827	**920.45**
	DCM	y = 1.936x-0.867	R^2 = 0.656	1072.69
	Ethyl acetate	y = 1.827x-0.563	R^2 = 0.599	1108.87
	Butanol	y =2.362x-1.523	R^2 =0.924	**577.62**
	Aqueous (***H_2O***)	y =1.777x-0.626	R^2 =0.623	1465.58
Hyoscyamus muticus CA00/43	Hexane	y =1.782x-0.625	R^2 =0.616	1434.05
	DCM	y =1.752x-0.862	R^2 =0.558	2217.64
	Ethyl acetate	y =1.863x-0.924	R^2 =0.858	1512.92
	Butanol	y =1.938x-0.780	R^2 =0.835	**960.41**
	Aqueous (***H_2O***)	y =1.679x-0.470	R^2 =0.588	1810.89
Calotropis procera CA04/02	Hexane	y =1.612x-0.288	R^2 =0.708	1907.20
	DCM	y =2.198x-1.753	R^2 =0.681	1181.24
	Ethyl acetate	y =2.075x-1.550	R^2 =0.670	1434.25
	Butanol	y =2.122x-1.533	R^2 =0.665	1198.67
	Aqueous (***H_2O***)	y =2.172x-1.589	R^2 =0.662	1180.46
Datura stramonium CA00/50	Hexane	y =2.348x-1.879	R^2 =0,988	**850.6**
	DCM	y =1.849x-1.132	R^2 =0,710	2071.99
	Ethyl acetate	y =2.031x-1.095	R^2 =0,699	1002.27
	Butanol	y =1.936x-0.851	R^2 =0,660	1052.47
	Aqueous (***H_2O***)	y =2.196x-1.549	R^2 =0,648	959.93

DCM: Dichloromethane

3.1. DISCUSSIONS

It is worthy to understand the biochemical elements that compose plants since this knowledge will be useful for facilitating the creation of complicated chemicals. Several studies have reported on this phytochemical analysis of different plants **(Pooja et al., 2015).** The active phytocomponents of the plants under inquiry were examined in the current study, and the results of the phytochemical test and qualitative evaluation of this plant indicated that:

- The phytochemical content of the ***C. procera*** leaves, stems, and fruit (pulps) extracts were found to be abundant. These extracts included terpenes, alkaloids, cardenolides, tannins, steroids, saponins, and flavonoids. Unsaturated sterols were not found in the stems, however. ***Mohammad Rowshanul Habib et al. (2016)***, in their research, verified this and detailed the harmful impact of ***Calotropis gigantea L.*** flower ethyl acetate on the stored grain pest ***T. castanium.*** Numerous statistical results, including the LD_{50}, ***95%*** confidence limit, and chi-square values, were obtained from the probit analysis of the mortality rate. In this study, the impact is shown using a ***Pergularia tomentosa*** butanol extract ***(577.62 min*** TL_{50}***, 95%).***
- A review of the literature confirmed the findings of the work of ***Bourmita et al.'s (2013)*** that was carried out in our laboratory. It showed that the mortality of ***Anacanthotermesochraceus***workers of termite was considerably impacted by the aqueous leaf extracts of these plants. The screening further showed that the LT_{50} of the ***C.procera*** leaf and stem extracts at ***5%*** concentration was LT_{50}***=54.24min*** and LT_{50}***=58.54min***, respectively, and that the ***P. tomentosa*** leaf extract at ***4%*** concentration was LT_{50} ***= 94.97 min.***
- According to ***Bader et al.'s (2021)*** study, root extracts of ***C. procera*** showed an important impact of ***50%*** ovicidal efficacy. This result correlates with extracts from ***64*** percent of the roots of Acorus calamus (Araceae) having ovicidal action. Also, it was greater than the extracts from ***Adhatoda vasica*** (15%), Vitex negundo (39%), and ***Dioscorea deltoidea (Dioscoreaceae)*** (14%), all at a comparable concentration (1.25%), when it came to ***Plutella xylostella (Lepidoptera: Plutellidae).***
- Previous research indicates that the amount of cardenolide in ***C. procera*** extracts may be connected to their antimicrobial action ***(Bader et al., 2021)***.
- Alkaloids, flavonoids, phenolics, tannins, and terpenoids were found in these plants, according to a phytochemical investigation ***(Mahmoudi et al., 2021)***.
- ***Ashamo et al. (2022)*** observed that flavonoids, oxalates, alkaloids, tannins, saponins, and phytates were among the phytochemicals found in the ethanolic agricultural wastes. The agricultural waste's insecticidal properties might be attributed to the existence of certain chemicals. These substances are secondary metabolites and the most significant bioactive components found in natural goods, having certain physiological effects on the body.

4. CONCLUSION

This study suggests that qualitative phytochemical screening of plant extract of***Datura stramonium*****,** ***Hyoscyamus muticus*****,** ***Pergularia tomentosa,*** **and** ***Calotropis procera*** supported the presence of bioactive compounds such as saponins, flavonoids, steroids, unsaturated sterols and terpenes, alkaloids unsaturated sterols, cardenolides and tannins, in toxic plants.

- The phytochemical screening demonstrated the presence of different types of phyto-compounds which could be responsible for the various pharmacological properties. Further studies are needed with these plants to evaluate their pharmacological potentials, isolate, characterize using advanced techniques of extraction, screening, identification, and isolation, elucidate the structures of the bioactive compounds responsible for their activities and they can be exploited in the future for further studies.
- The treatment of ***Triboliumcastanium***by the butanol extractof ***P. tomentosa***and ***D. stramonium*** under controlled conditions revealed their insecticidal properties. This toxic effect is due to the presence of one or many active compounds. Therefore, the identification of compounds responsible for these activities and toxicological studies to determine their mode of action are necessary to confirm their performances as bio-insecticide alternatives to chemical control.

- Spononins, flavonoids, steroids, terpenes, alkaloids, cardenolides, and tannins have been observed in ***D. stramonium*** leaves. However, there were no unsaturated sterols. ***Chentem Williams et al. (2015)*** reported that the ***D. stramonium*** ethanol leaf extract included alkaloids, flavonoids, terpenoids, steroids, and tannins.
- Saponins, flavonoids, steroids, terpenes, alkaloids, cardenolides, and unsaturated sterols have been identified in the stems and whole fruits. The research results indicated that the whole fruit was devoid of cardenolides and tannins.
- Saponins, flavonoids, steroids, terpenes, alkaloids, cardenolides, and tannins were detected in all parts of ***H. muticus*** leaves, stems, and entire flowers. Unsaturated sterols did not exist in the leaves or whole flowers ***(Table 2)***. Our findings correspond with those of **Mohd et al. (2017),** who reported that all fractions of ***H. muticus*** ethanol extracts contain triterpenes, sterols, and alkaloids after an initial phytochemical screening of the leaf, flower, and stem fractions. Besides, leaf and floral extracts include flavonoids.
- Spononins, flavonoids, steroids, terpenes, alkaloids, unsaturated sterols, and cardenolides had been identified in the leaves and stems of ***P. tomentosa***; tannins did not exist in the stems ***(Table 3)***.
- Most of the examined plants contained flavonoids, whereas glycosidic flavonoid molecules were found in all plant materials. Heterosidic flavonoids, on the other hand, are entirely absent from all plant components, with the exception of ***H. muticus*** stems.
- Secondary metabolites varied across the plant sections; pharmacologists and physiochemists will find the current study's results useful in identifying novel active chemicals.
- The detrimental effects of various leaf extracts from four plants (***P. tomentosa, H. muticus, C. procera, and D. staramonium***) on adult ***T. castanium*** were examined in the present study. After five days of exposure, the butanol extract of ***P. tomentosa*** took ***577,62*** minutes, the hexane extract of ***P. tomentosa*** took ***920,45*** minutes, the hexane extract of ***D. staramonium*** took ***850,6*** minutes, and the butane extract of ***H. muticus*** took ***960,41*** minutes. The presented results were derived from the probit analysis of mortality rate, several statistical data (TL_{50}), and regression coefficient (R^2) values.
- Important characteristics of toxicological inference TL_{50}were identified by the research results, and they were found in the TL_{50}values of ***D.staramonium's*** hexane extract and ***P. tomentosa's*** butanol extract. ***Table 4*** displays the TL_{50}values for the various extracts over a ***5-day*** period. With a $TL_{50}$of ***577.62*** minutes, our investigation revealed that the butanol extract of ***P. tomentosa*** produced toxic effects against mature ***T. castanium.***

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