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PHYTOCHEMICAL SCREENING, ANTIOXIDANT ACTIVITY, ANTI-INFLAMMATORY ACTIVITY ANTIMITOTIC ACTIVITY AND POTENTIAL OF *TEPHROSIA MAXIMA* PODS

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ABSTRACT

Background: *Tephrosia* is a large tropical and sub-tropical genus belonging to the family Fabaceae. *Tephrosia maxima* is reported to be rich in phenols and flavonoid content. The folklore of Andhra Pradesh uses the plant for anti-inflammatory condition, as anti-ulcer, anti-oxidant and anti cancer/Cytotoxic agents. **Aim**: The present study aims to assess the antioxidant activity, anti-inflammatory activity and antimitotic activity of the chloroform and methanolic extract of the Pods of the *Tephrosia maxima in vitro*. **Materials and Methods**: To the extracts preliminary phytochemical screening was performed initially followed by estimation of *in vitro* anti-oxidant activity by using DPPH measuring the absorbance. The *in vitro* bioassay consisted of assaying the effect of the extracts against denaturation of protein (egg albumin) and measuring the absorbance. Antimitotic activity of the extract was determined by green gram seed phytotoxicity assay and cytotoxicity by brine shrimp nauplii lethality assay. **Results:** The plant extracts revealed the presence of Polyphenols, Alkaloids, Flavonoids, Anthraquinones, Coumarins, Steriods, Lignans, Tannins and Terpenoids. The *in vitro* anti-oxidant activity have shown chloroform extract more potent at even lowest concentrations when compared to methanolic extract. The *in vitro* study of chloroform extract of *Tephrosia maxima* demonstrated that at 150 µg/ml concentration a better anti-inflammatory activity was exhibited which is more than the methanolic extract. Similarly in the case of *in vitro* bioassay of antimitotic and cytotoxicity studies have shown very good activity in case chloroform extract when compared to methanolic extract. **Conclusion:** The chloroform extract of *Tephrosia maxima* pods have showed potent *in vitro* antioxidant, anti-inflammatory, antimitotic and cytotoxic activities when compared to methanolic extract. It may be due to presence of phytochemicals like polyphenols, Alkaloids, Flavonoids and terpenoids

Keywords: Polyphenols, Alkaloids, Flavonoids, Anthraquinones and Coumarins.

INTRODUCTION

Tephrosia maxima or Galega maxima.L is a prostate herb belonging to the family Fabaceae and are legumes in nature. The plant is constituted with 6-9 pairs of leaflets. They are thin coriaceous, oblanceolate, entire, truncate-obtuse and mucronate. The flowers are pink in colour and are arranged in leaf opposed pseudoracemes. Sepals are appressed, pubescent, standard sericeous and obovate. The plant is distributed in wastelands and dry deciduous forests. The flowering and fruiting of the plant is usually throughout the year[1,2]. 7, 8 –methylenedioxy isoflavone, maxima isoflavone A, B, H, J and T were isolated from Tephrosia maxima [2,3].

Variety of problems have been observed when quality aspect is considered. The reason may be quoted with respect to the nature of herbal ingredients and variety of secondary metabolites present in them. The reason can be extended to the chemical profile of herbs as various conditions including intrinsic and extrinsic factors also affect in one way or the other[4]. Greater part of the crude drugs arrive from wild sources and is usually collected by poor, illiterate tribal devoid of much awareness to botanical identification and authentication. As the natural products are multifaceted due to their heterogeneous composition the standardization is often a complex assignment. To ascertain the superiority of herbal medicines, appropriate control of starting material is extremely crucial. The primary pace for assuring quality of starting material is authentication which may be followed by developing numerical values of standards for assessment. Pharmacognostical parameters for unproblematic recognition like microscopy, physico chemical analyses, fluorescence analysis are few of the vital etiquette for standardization of herbals[5]

MATERIALS AND METHODS

Preparation of *Tephrosia maxima* pods extract

Tephrosia maxima pods were collected between (July to August), and dried at ambient temperature. Pods were milled to a fine powder using an electrical mill, and then stored in the dark in closed containers until required. To obtain the pods extract, 100g of powdered pods were macerated in 1000 ml of absolute methanol for 48 h with agitation.

Phytochemical analysis

The methanol extract was submitted to phytochemical analysis for secondary metabolites identification using the phytochemical methods, which were previously described. In general, tests for the presence or absence of phytochemical compounds involved the addition of an appropriate chemical agent to the preparation in a test tube. The mixture was then vortexed. The presence or absence of saponins, flavonoids, tannins, and alkaloids etc., were subsequently detected.

Antioxidant[6]

2, 2-Diphenyl-1-picryl-hydrazyl (DPPH) radical is a relatively stable compound with a peak absorbance at 517 nm (A517) [6,7]. It has an unpaired valence electron at one atom of a nitrogen bridge. The reaction of a DPPH radical along with a variety of antioxidants acting as donors of an electron and/or a hydrogen radical leads to the formation of 1,1-diphenyl-2-picrylhydrazine and a loss of A517,[6,8]. Antioxidants can also form colorless unstable adducts with a DPPH radical that may further release modified antioxidants capable of reducing another DPPH radical molecule, thus augmenting the decline at A517. Therefore, the decrease at A517 after the addition of a tested antioxidant or a mixture of various plant antioxidants could be a slow process entailing a relatively extensive time in its complete development[6]. Consequently, the decline at A517 in relation to an antioxidant-induced decomposition of a DPPH radical (the so-called DPPH test) is monitored over a 20-60 min incubation period [9-10]. However, in the case of hydrophilic antioxidants, a mixture of alcohol with either an acidic acetate buffer or water is applied[11, 12]. Under these conditions, a DPPH radical may undergo spontaneous decomposition, necessitating parallel control experiments in every tested sample. This approach is based on a disputable assumption concerning the additive effect of water and an antioxidant on the decomposition of a DPPH radical likely being a source of unpredictable errors.

The assay sensitivity, linearity, repeatability, the effect of temperature, storage duration, the addition of various antioxidants on the decomposition of a DPPH radical by chloroform and methanolic extract of *Tephrosia maxima* pods was determined. Finally, associations between the results of the DPPH test and other photometric methods for the determination of the total antioxidant capacity. The DPPH assay is a general test used to evaluate the antioxidant capacity of plant extracts. For this study, methodology of Hatano et al[13] will be used for estimation of radical scavenging activity of plant samples at various concentrations (0, 50, 100, 150, 200, 250,300 and 350 µg mL-1) were mixed with 5 mL of DPPH solution (0.05 mM in methanol) and incubated for 30 min at room temperature.

Absorbance was determined at 517 nm using DPPH solution as blank. Ascorbic acid was used as standard antioxidant.
The percentage inhibition of DPPH (I%) was calculated using the following formula

$$I(\%) = 100x\ (A0-As)\ /A0$$

Where,
A0 absorbance of control
As absorbance of test sample

Anti-inflammatory activity
Anti-inflammatory bioassay in vitro
According to previously reported protocol,[14,15] The reaction mixture consisted of 0.2 ml of egg albumin (from fresh hen's egg), 2.8 ml of phosphate buffered saline (pH 6.4) and 2 ml of varying concentrations of the test extract, by which the concentrations (µg/ml) became 10, 25, 50, 75, 100, 150. Similar volume of double-distilled water served as control. Then the mixtures were incubated at 37°C ± 2°C in a biological oxygen demand incubator for 15 min and then heated at 70°C for 5 min. After cooling, their absorbance was measured at 660 nm (LAB INDIA Spectrophotometer) by using vehicle as blank. Diclofenac sodium and rumalaya forte at the final concentrations (µg/ml) of 50, 100, 150, 200, 250 and 500 were used as reference and traditional/herbal drugs respectively and treated similarly for determination of absorbance. Test extracts were chosen such that, they remained the nearest possible to the standard therapeutic mode.
The percentage inhibition of protein denaturation was calculated by using the following formula:

$$\%\ \text{inhibition} = 100 \times ([Vt/Vc] - 1).$$

Where,
Vt = absorbance of test sample, Vc = absorbance of control.

Antimitotic activity
Bioassay: Antimitotic activity of the extract was determined according to Turker *et al*[16]., using wheat seeds at germination and early seedling growth stage, cytotoxicity by brine shrimp nauplii lethality assay according to Meyer *et al*[17].,. Each set of experiment was replicated three times and their mean values were taken.

RESULTS AND DISCUSSION
Phytochemical screening of *Tephrosia maxima* pods
The results of our preliminary phytochemical analysis of *Tephrosia maxima* pods extracts were given in Table-1, which revaled the presence of seven known compounds as: Polyphenols, Alkaloids, Flavonoids, Anthraquinones, Coumarins, Steriods, Lignans, and Tannins in *Tephrosia maxima* pods methanolic extract. While *Tephrosia maxima* pods chloroform extract have shown the presence of all previously tested compounds except the Terpenoids. (Table 1: Phytochemical constituents of *Tephrosia maxima* pods)

Antioxidant activity
The chloroform and methanolic extracts of *Tephrosia maxima* pods were studied for antioxidant activity. DPPH is a purple stable free radical with an adsorption band at 517 nm and can be reduced to yellow by accepting electrons from antioxidants[18]. Fig 1 depicts the ability of different concentrations (10-350 µg/mL) of the plant extracts to scavenge DPPH free radicals compared to the ability of ascorbic acid. From the table Ascorbic acid presented the lowest IC50 value (37.5 µg/mL). As per Arrigoni *et al.,*[19], efficient biological antioxidants are supposed to: (a) exist in good quantities in the cell, (b) react with several free radicals, (c) be ideal for regeneration other than just reacting with free radicals. These properties are typical for ascorbic acid, making it a good antioxidant for the cells of almost all aerobic species. Among the two plant extracts, the radical scavenging activity of chloroform was almost equivalent to that of ascorbic acid at the concentration of 150 µg/mL and above. (Fig. 1)

Anti-inflammatory activity
Choloform extract of *Tephrosia maxima* pods at the dose of 150µg/ml exhibited an anti-inflammatory activity that became significant ($P < 0.01$) with a 65% more inhibitory effect than the stem extract as presented in Table 2.
Denaturation of tissue proteins may be the cause behind the production of auto-antigens in certain arthritic diseases. So it may be said that tissue protein denaturation is a marker for inflammatory and arthritic diseases.[20] Agents that can prevent protein denaturation, therefore, would be possible candidate for anti-inflammatory drug development. With this idea in mind, the in vitro test was done as a preliminary screen to check presence of anti-inflammatory property. In the present study, the protein denaturation bioassay was selected for in-vitro assessment of anti-inflammatory property of chloroform extract of *Tephrosia maxima* pods with a wide range of dose concentrations. All the results were statistically significant ($P < 0.01$).
During preliminary phytochemical screening of the crude extracts of both the choloform and methanol, important therapeutic principles such as alkaloids, saponins, flavonoids, tannins, terpenoids and polyphenols etc., were

detected. Therefore, the current findings can be attributed to these groups of chemical compounds. Further study is need on these plant extracts to find the exact mechanism of action for its pharmological properties over its anti-inflammatory effects. (Table 2: Influence of Extracts of Tephrosia Maxima against Protein Denaturation)

Antimitotic activity

Inhibition of cell division is a measure of the antimitotic activity of chemical compounds. Growth inhibition test provides a way of detecting the antimitotic activity of chemical compounds under laboratory conditions.

In the present study, model of green gram seed phytotoxicity assay was used to evaluate growth stimulation or inhibition properties of the choloform and methonlic extracts of *Tephrosia maxima* pods by taking wheat seed as test material. The seed germination counts taken on 5th day following soaking were 50 and 25 at 100 ppm and 150 ppm of choloform extract, respectively (Fig. 2) and 10 and 60 at 100 ppm and 150 ppm of methanolic extract, respectively (Fig. 3). The root lengths measured after the same time intervals were 30mm and 0.5mm at 100 ppm and 150ppm of choloform extract 30mm and 38mm of methanolic extract, respectively.

All these indicate that inhibition of the protrusion of plumule and radicle through the seed hilum at germination and the elongation of the root at seedling growth stages were due to the suppression of mitotic cell division and higher doses were more effective in inhibiting such growth activities, possibly due to high content of secondary metabolites.

The **cytotoxic activity** of the choloform and methonlic extracts of *Tephrosia maxima* pods was determined by the brine shrimp nauplii lethality bioassay using six concentrations of the extract ranging from 10 to100 µg/ml, each with 10 nauplii, which died progressively in greater number with the increase of the concentration of the extract and exposure time (up to 24 hours) at the rate of 0.025, 0.1, 0.2, 0.4, 0.8 and 0.975% (Table 3).

The cytotoxicity of plant material would likely indicate the presence of antitumor compounds in plant extract Martin-Cordero *et al*[21].,. According to Rieser *et al*[22]., crude extracts resulting an LC50 value of less than 250 µg/ml could be considered significantly active and potential for further investigation. The choloform extract *Tephrosia maxima* pods exhibited LC50 value less than 150 µg/ml indicating its potential as a source of anticancer agent. The choloform extract of the present work exhibited the inhibitory activity in a dose dependent manner.

Table 1: Phytochemical constituents of *Tephrosia maxima* pods

	Chloroform extract	Methanolic extract
Alkaloids	+	+
Flavonoids	+	+
Saponins	-	-
Coumarins	+	+
Anthocyanins	-	-
Anthraquinones	+	+
Terpenoids	+	-
Polyphenols	+	+
Tannins	+	+
Lignans	+	+
Steriods	+	+

Table 2: **Influence of Extracts of Tephrosia Maxima against Protein Denaturation**

Concentration(µg/ml)	%Inhibition (chloroform extract)	%Inhibition (methanolic extract)
Control	-	-
10	5.88 (0.04)*	1.96 (0.01)*
25	7.84 (0.08)*	3.92 (0.03)*
50	17.64(0.13)*	7.84 (0.05)*
75	56.86(0.38)*	27.45(0.22)*
100	154.9(1.16)*	66.66(0.71)*
150	435.29(3.49)*	150.98(1.28)*

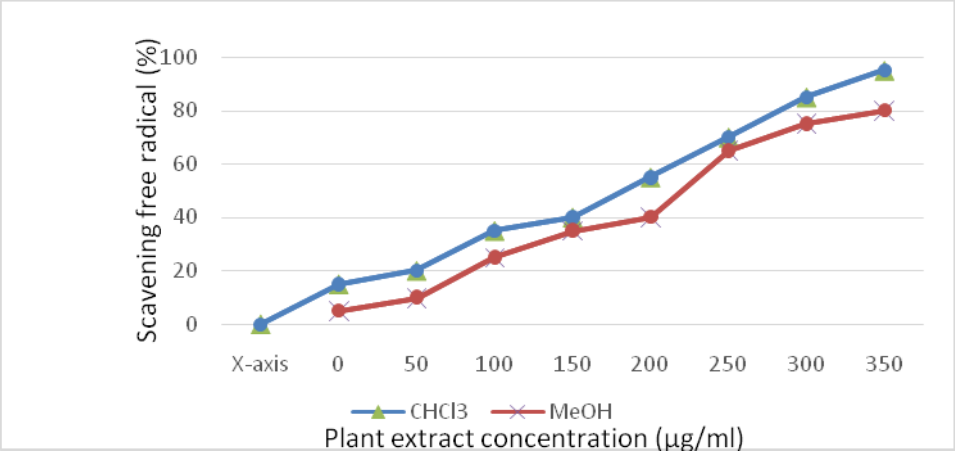

Fig. 1:

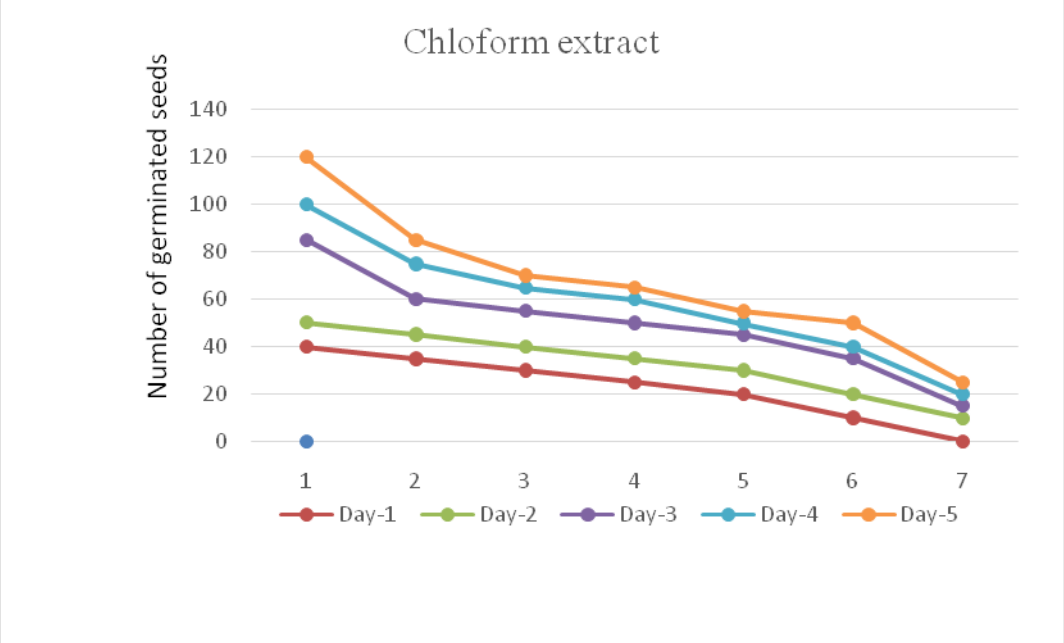

Fig. 2: Effects of chloroform extract of *Tephrosia maxima* pods on germination of green gram seeds

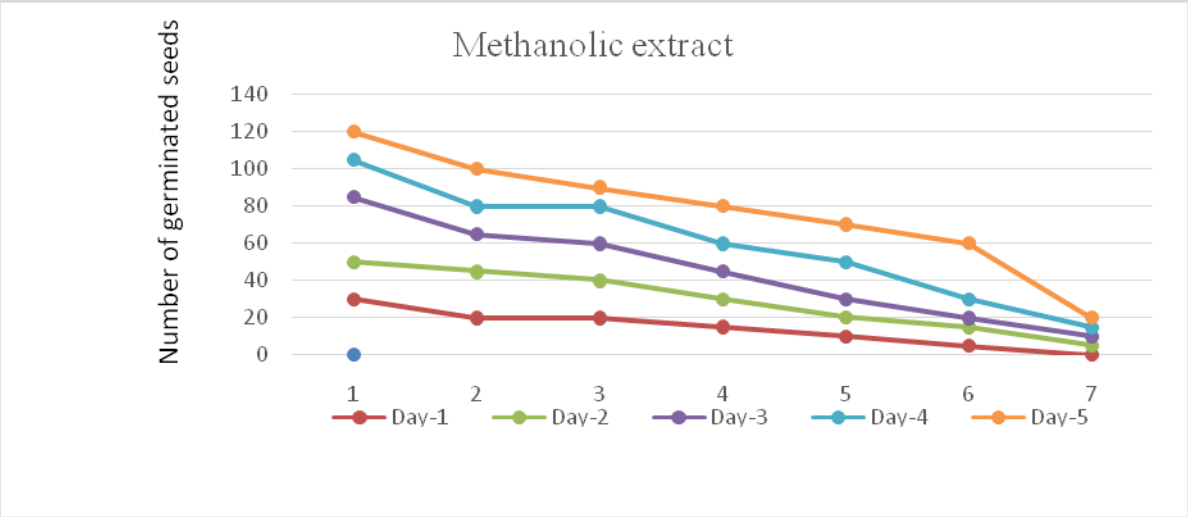

Fig. 3: Effects of Methanolic extract of *Tephrosia maxima* pods on germination of green gram seeds

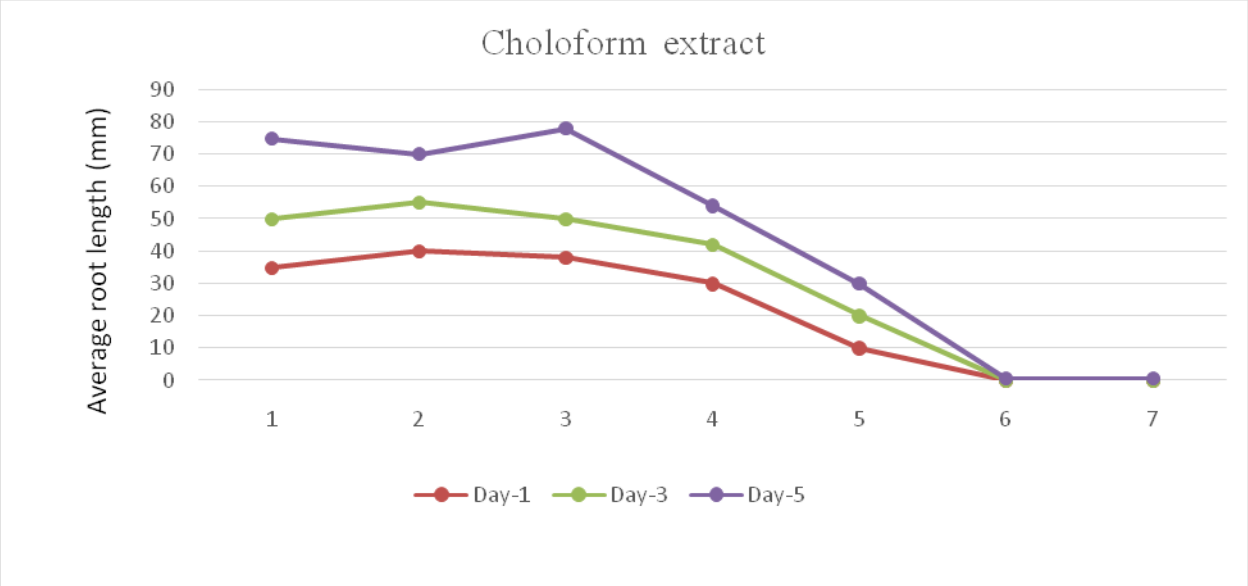

Fig. 4: Effects of choloform extract of *Tephrosia maxima* pods on rate of root growth in length of green gram seedlings

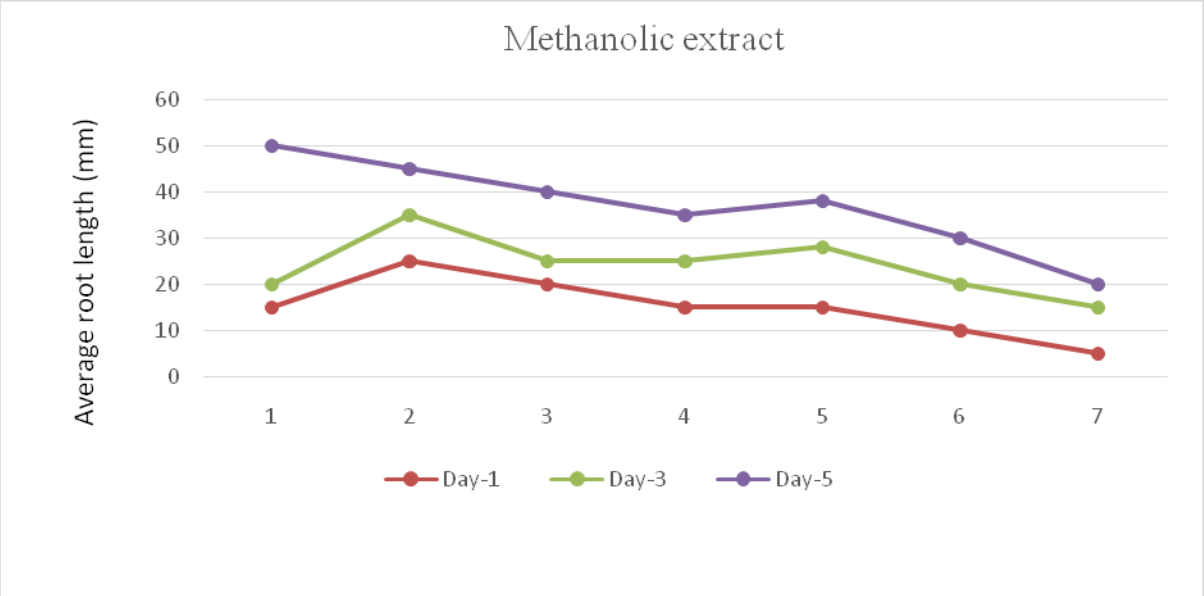

Fig. 5: Effects of methanolic extract of *Tephrosia maxima* pods on rate of root growth in length of green gram seedlings

Table 3: Cytotoxic effect on *Artemia salina* nauplii due to chloroform extract of *T. maxima*

Dose (µg/ml)	Log dose	Total nauplii	Survived nauplii	Dead nauplii	Lethality (%)	Actual (%)	Probit
10	1	10	10	0	0	0.025	3.04
25	1.3	10	9	1	10	0.1	3.72
50	1.4	10	8	2	20	0.2	4.16
75	1.7	10	6	4	40	0.4	4.75
100	1.9	10	2	8	80	0.8	5.84
150	2	10	0	10	100	0.975	6.96

CONCLUSION

Hence, it may be concluded from the present findings that the choloform extracts of *Tephrosia maxima* pods have significant antioxidant, anti-inflammatory and antimitotic activity and cytotoxicity in vitro systems. Further research to investigate and find out the responsible components may be undertaken or responsible for and they may be incorporated into existing antioxidant, anti-inflammatory and antimitotic activity and cytotoxicity herbal compositions to improve their safety efficacy by furthermore scientific studies wherever needed.

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