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BIO SAFETY AND ANTI INFLAMMATORY ACTIVITY OF METHANOLIC EXTRACT OF *TRICHODESMA INDICUM* LINN (R.BR) AS A WEED

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ABSTRACT

Trichodesma indicum Linn R.Br is a common weed in *Boraginaceae* family. The biosafety and anti inflammatory activity of the methanolic extract was screened by means of using membrane stabilization capacity towards the newly hatched Brine shrimp larvae as well as Human HRBCs suspension respectively. The study showed that it is biologically safe (LD_{50} 891.25mcg/mL) as well as it has a good anti inflammatory activity (71.21 ± 2.06% at 88.89mcg/mL).

Key Words: *Trichodesma indicum,* Brine shrimp larvae, Cytotoxicity.

INTRODUCTION

Trichodesma indicum Linn is an erect, spreading, branched, annual herb, about 50 centimeters in height from *Boraginaceae* family. Leaves are stalkless, lanceolate, 2 to 8 centimeters long. Flowers occur singly in the axils of the leaves. Calyx is green, hairy, and 1 to 1.3 centimeters long, with pointed lobes. Corolla is pale blue, with the limb about 1.5 centimeters in diameter, and the lobes pointed. Fruit is ellipsoid, and enclosed by the calyx. Distributed in Iran, India, and Mauritius.The main constituents are ß-sitosterol and phenolics, catechin and gallic acid. Seed oil contains oleic, linoleic, palmitic, stearic, and linolenic acid [1].

MATERIALS AND METHODS

Collection of plant and preparation of extract

The healthy whole plants of *Trichodesma indicum* Linn R.Br was collected in and around Madurai medical college campus and

authenticated by Dr Stephan., Ph.D., Reader in Botany, The American College. Madurai Tamilnadu and a voucher specimen were kept in the Department of Pharmacognosy, College of Pharmacy, Madurai Medical College. Madurai in the reference no: PCG 001/2010. The plant materials were cleaned, washed and dried under shade and pulverized to get 40# size particles and then extracted with petroleum ether followed by Methanol for 72hrs respectively. Methanolic extract was filtered and concentrated under vacuum using Rota evaporator. The dried extract was used for the further phytochemical as well as biological studies.

Bio safety studies

The modified method adopted by Solis *et al.,* 1992 and Omale *et al.,* 2008 & 2009 [2] were used for determining the bio safety of the methanolic extract by inhibitory activity on *Artemia salina* (brine shrimp larvae) cytotoxicity .

Procedure

The shrimp eggs were hatched in sea water media at 28^{o}C for 48h in a hatching tray. The methanolic extract of *T. indicum* was suitably dissolved in 0.1% Tween 80 to get concentrations ranging from 200-1000µg/mL. The newly hatched brine shrimps (10 in number) were transferred into vials containing 4.5mL of sea water and 0.5mL of different concentrations of extract. The vials were incubated for 24 hours. A set of 3 vials for each concentration was incubated to make the overall tested organisms of 30 for each concentration of the extract. A set of three vials without the sample and another set of three vials with potassium dichromate [3] served as negative and positive controls respectively. The living brine shrimps were counted under a hand magnifying lens. The percentage lethality versus log concentration was plotted and Y=50 is substituted in the resulting linear equation to obtain the X value. The antilog of X was then the LC50 (conc. of 50 lethality) value [4] Table 1.

***In vitro* membrane stabilisation study**

The method of Sadique et al (1989) and modified by Oyedapo and Famurewa (1995) and Oyedapo *et al.,* (2004) was employed in the membrane stabilizing activity assay. When RBCs are subjected to heat and treatment with hypo saline they release hemoglobin which has a maximum absorbance at about 560nm. The capacity of the extract to reduce hypo saline and heat induced lysis is basis of the assay.

Procedure

The assay mixture consisted of 2mL of hypo saline and 1mL of phosphate buffer and varying volumes of the extract (0.1 to 0.5mL) and 0.5mL of 10% v/v HRBC suspension in isosaline, then the final volume were made up with isosaline up to 4.5mL. The control was prepared as mentioned above except the drug was omitted, while drug control was also prepared similarly but without HRBC suspension. The reaction mixture was incubated at 56ºC for 30min in a water bath, then the tube was cooled under running water. Then the absorbance of the released hemoglobin was measured at 560nm using Shimadzu UV Visible spectrophotometer, Model 1800. Diclofenac 50µg/mL was used as a reference standard [5-7]. The percentage membrane stabilization activity of the compounds was determined by the formula Table 2.

% membrane stabilization = [$A_{control}$ - (A_{test} - $A_{product\ control}$)] / $A_{control}$ x 100

$A_{control}$ = Absorbance in control

A_{test} = Absorbance in test

$A_{product\ control}$ = Absorbance in product control

Determination of scavenging activity against hydrogen peroxide

The radical scavenging activity against hydrogen peroxide of plant extract was determined by using the method of Ruch *et al* .The principle is based the capacity of the extracts to decompose the hydrogen peroxide to water [8].

Procedure

The methanolic extract of *Trichodesma indicum* was dissolved in ethanol to get a stock solution of 1mg/mL. Varying quantities of the stock solution (22.22-177.78µg/mL) were added to 3.8mL of 0.1M phosphate buffer solution (pH 7.4) and then mixed with 0.2mL of hydrogen peroxide solution. The absorbance of the reaction mixture was measured at 230nm after 10min by using Shimadzu UV Visible spectrophotometer, Model 1800. The reaction mixture without sample was used as blank. Ascorbic acid was used as standard [9]. The percentage inhibition of hydrogen peroxide was calculated using the formula [Table 3 and Fig. 1].

Percentage inhibition = ($A_{control}$ - A_{sample}) / $A_{control}$ x 100.

Total antioxidant activity by Phosphomolybednum method

Procedure

An aliquot of 0.3ml of different concentrations of sample solutions was combined with 2.7ml of the reagent solution (H_2SO_4, sodium phosphate and ammonium molybdate). In case of blank, 0.3mL of methanol was used in place of sample. The tubes were incubated in a boiling water bath at 95°C for 90min. After the samples had cooled to room temperature, the absorbance of the aqueous solution of each was measured at 695nm against blank using Shimadzu UV Visible spectrophotometer, Model 1800. The standard vitamin C was treated in a similar manner. The antioxidant activity was expressed as equivalents of Vitamin C (µg/g) [9] [Table 4 and Fig.2].

RESULTS

Bio safety studies

Table 1: Inhibitory effects of methanolic extract of *T. indicum* on *Artemia salina*

Name of the extract/ Standard	Conc. in µg/mL	Log concentration	LC_{50} in µg/mL
Potassium dichromate	6.25	0.7959	**29.245**
	12.5	1.0969	
	25	1.3979	
	50	1.6990	
	100	2.0000	
Methanolic extract	200	2.3010	**891.25**
	400	2.6021	
	600	2.7781	
	800	2.9031	
	1000	3.0000	

In vitro membrane stabilization study

The results obtained for the *in vitro* membrane stabilization study are presented in **Table 2.** The extract at a concentration of 88.89µg/mL had a percentage membrane stabilization of 71.21 ± 2.06 which was more than that of the standard diclofenac at 50µg/mL.

Table 2: Percentage of membrane stabilization of methanolic extract of *T. indicum*

S.No.	Treatment	Conc. in µg/mL	Percentage of membrane stabilization
1.	**Diclofenac**	50	48.60 ± 0.90
2.	**Methanolic extract of *T. indicum***	22.22	7.52 ± 0.91
		44.44	19.36 ± 0.40
		66.67	35.08 ± 1.14
		88.89	71.21 ± 2.06
		111.11	50.86 ± 1.21

***mean of three readings ± SEM**

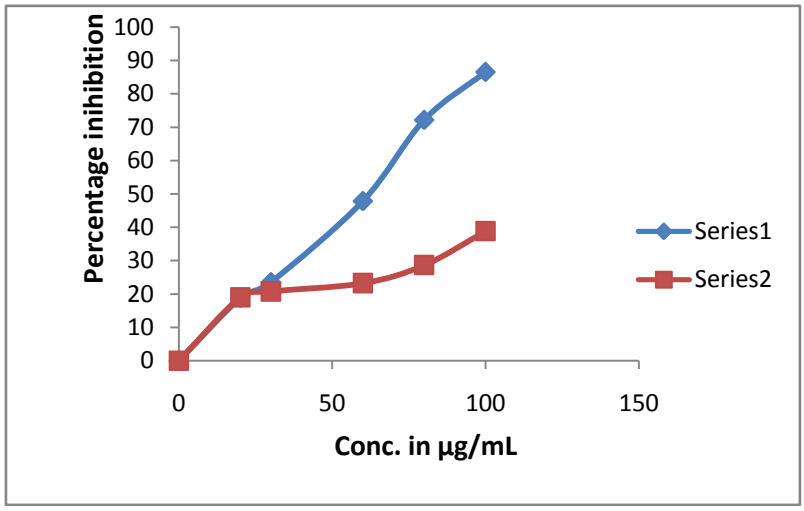
HYDROGEN PEROXIDE SCAVENGING ACTIVITY

Table 3: Percentage inhibition of hydrogen peroxide by methanolic extract of *T. indicum*

S.No.	Conc. in µg/mL	Percentage inhibition	
		Methanolic extract of *T. indicum*	Ascorbic acid
1	20	18.31 ± 0.62	18.81
2	40	20.77 ± 0.82	23.52
3	60	23.48 ± 0.13	47.85
4	80	30.28 ± 0.90	72.18
5	100	38.99 ± 0.10	86.56
	IC_{50}	**156.67 µg/mL**	

***mean of three readings ± SEM**

Fig. 1: Percentage inhibition of hydrogen peroxide by methanolic extract of *T. indicum* and ascorbic acid



Total antioxidant activity

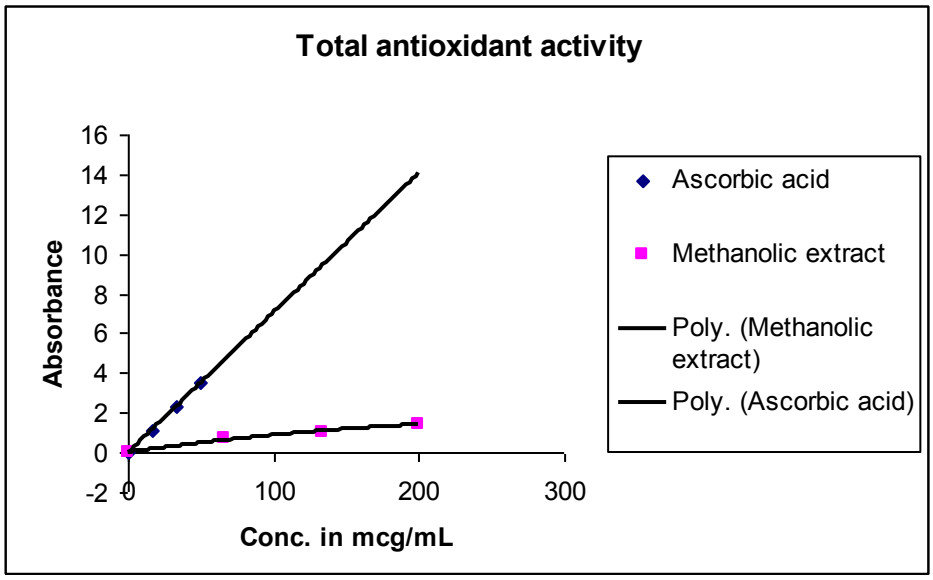
The results obtained for the total antioxidant activity of methanolic extract of *T. indicum* and standard ascorbic acid are tabulated in **Table 4** and **Fig. 2**.

Table 4: Total antioxidant activity of methanolic extract of *Trichodesma indicum*

S.No.	Conc. in µg/mL	Abs. of standard ascorbic acid*	Conc. in µg/mL	Abs. of methanolic extract*
1	**16.67**	1.165 ± 0.006	66.67	0.683 ± 0.012
2	**33.33**	2.350 ± 0.001	133.33	1.043 ± 0.007
3	**50**	3.515 ± 0.004	200	1.428 ± 0.054

***mean of three readings ± SEM**

Fig 2: Total antioxidant activity of methanolic extract of *Trichodesma indicum*



DISCUSSION

Cytotoxicity assay

From the table it can be seen that the extract possessed very low cytotoxicity to brine shrimp with LC_{50} of 891.25 which ensures the biosafety of the extract. The brine shrimp assay is a rapid, inexpensive and an efficient assay which will a good correlation with antitumour activity.

Hydrogen peroxide scavenging activity

The scavenging of hydrogen peroxide by ascorbic acid and methanolic extract of *T. indicum* after incubation increased with increase in concentration. Hydrogen peroxide by itself is not very reactive [10] but it is capable of generating a highly reactive hydroxyl radical through the Fenton reaction [11& 12].

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH + OH^-$$

Hence the scavenging of hydrogen peroxide is an important defence mechanism [13]. Hydrogen peroxide is decomposed to water by the transfer of electron as given in the equation below [14]

$$H_2O_2 + 2H^+ + 2e^- \rightarrow 2H_2O$$

The inhibitory effect of the extract on hydrogen peroxide may be attributed to the phenolic compounds present due to their electron donating capacity.

Addition of hydrogen peroxide to cells in culture can lead to transition of metal ion dependent hydroxyl mediated oxidative DNA damage. Levels of hydrogen peroxide at or below about 20-50mg seem to have limited cytotoxicity to many cell types. Since phenolic compounds present in the plant extract are good electron donor, they may accelerate the conversion of hydrogen peroxide to water.

Total antioxidant assay

Hydrogen and electron transfer from antioxidant analytes to Mo (VI) complex occur in the phosphomolybdenum assay. The transfer of electron from the analyte depends on the structure of the antioxidant. The phosphomolybdenum method usually detects antioxidants such as phenolics, α-tocopherols and carotenoids [15]. The absorbance increases as the concentration of the extract increases which shows a good reducing capacity of the extract. A concentration of 200µg/mL of the extract showed an absorbance of 1.428±0.054. A linear regression equation y = 0.07038x – 0.0021 was formed for the standard ascorbic acid and the antioxidant activity was expressed as 101.6 mg equivalents of ascorbic acid /g of the extract.

***In vitro* membrane stabilization study**

Lysosomes are intracellular particles which contain most of the lytic and digestive enzymes of the tissue. The rupture of the lysosomes results in injury or death to surrounding tissues and also acute inflammation. The membranes of lysosomes and erythrocytes are destroyed by similar agents; hence a test was developed to measure the ability of compounds to stabilize erythrocyte membrane to heat hemolysis. RBCs when exposed to various injurious substances such as methyl salicylate, phenyl hydrazine, and hypotonic medium or over heat will cause lyses of membrane accompanied by heamolysis and oxidation of hemoglobin [16]. RBCs membranes are easily susceptible to free radical mediated lipid per oxidation by break down of biomolecules. Due to it has rich source of iron and high oxygen partial pressure. RBCs membranes are similar to lysosomal cells. All NSAIDs inhibited hemolysis while other type of compounds had no effect [17]. Hence prevention of hypotonic and heat mediated RBCs membrane lysis taken as measure of anti inflammatory activity of drugs.

A study has reported that the flavanoids exert membrane stabilizing effect on lysosomes both *in-vitro* and *in vivo* in experimental animals [18 & 19]. Another report has suggested that tannins and saponins have the ability to bind cations and other bio-molecules and are able to stabilize the erythrocyte membrane [20& 21]. Investigations have also revealed that various herbal formulations are capable of stabilizing red blood cell membrane and exert anti-inflammatory activity [22& 23].

From the **Table 4**, it can be seen that extract is highly potent on human erythrocyte and thus adequately protecting

it against heat and hypotonicity induced lysis. The phytochemical analysis showed that the methanolic extract of *T.indicum* has flavanoids, tannins and saponins. Hence the HRBC membrane stabilizing capacity may be due to the presence of the above mentioned constituents which will prevent the oxidation of hemoglobin and also its antioxidant property.

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