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Research Article

THE INHIBITORY EFFECT OF SOME INDIAN PLANT EXTRACTS ON THE ANILINE HYDROXYLASE

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ABSTRACT

Objective: The present study was carried out to evaluate the *in vitro* inhibitory activity of whole plants of ethanol extract of *Euphorbia hirta* Linn, methanol extract of leaves of *Euphorbia nerrifolia* Linn, Ethanol extract of stem bark of *Euphorbia tirucalli* Linn on Phenobarbitone induced aniline hydroxylase enzyme.

Materials & Methods: Different concentrations of the all extracts of Euphorbia plant (600,500,400,300,200,100g/ml) were subjected to study aniline hydroxylase inhibitory activity against Phenobarbital (80mg/kg) induced in rat orally 5 days then remove liver and by measuring the absorbance at 630 nm.

Results: On in vitro experimental study of whole plant of *Euphorbia* hirta L showed 50% inhibitory concentration of aniline hydroxylase was potent as compared the stem bark extract of *Euphorbia* tirucalli Linn and methanol extract of leaves of *Euphorbia nerrifolia* Linn. All extracts showed a dose dependant inhibition of aniline hydroxylase.

Conclusion: The ethanolic extracts of *Euphorbia hirta L, Euphorbia tirucalli* Linn*, Euphorbia nerrifolia* Linn were showing potent inhibitory activity of enzyme aniline hydroxylase.

Keywords: *Euphorbia hirta Linn,* enzyme aniline hydroxylase, *Euphorbia tirucalli* Linn**,** *Euphorbia nerrifolia* Linn

INTRODUCTION

Several species of the genus *Euphorbia* (Euphorbiaceae) have been tested for their efficiency as antiviral and Antitumour agents, partly based on information concerning plants that have traditionally been used as medication to treat various human diseases[1]. The family Euphorbiaceae is the sixth largest and one of the most diversified families of angiosperms, consisting of about 300 genera and over 8000 species [2].

Euphorbia hirta L. is a medicinal, rhizomatous herb distributed in Southern Western Ghats of India and found especially on roadsides and wasteland. In India it is used to treat worm infestations in children and for dysentery, gonorrhoea, jaundice, pimples, digestive problems and tumours. The aerial parts of plant are well investigated for chemical information like quercitrin and quercitol, Euphorbins A, B, C, D, E, Gallic acid, myricitrin., This plant was reported in various pharmacological activities like Anti-inflammatory activity, Antibacterial activity, Anticancer activity, Diuretic activity etc[3,4].

Euphorbia neriifolia Linn is a shrubby, erect, branched, fleshy, cactus like plant, 2 to 4 meters high, the trunk and older branches being grayish and cylindric. The leaves arise from the sides of wings towards the end of the branches, are fleshy, oblong-obviate, 5 to 15 centimeters long, or in young plants somewhat longer, painted or blunt at the tip. The expressed juice of the leaves is reported as very effectual in relieving the paroxyms of spasmodic asthma. The leaves are considered diuretic [5].

Euphorbia tirucalli L. (E. tirucalli), commonly known as aveloz or pencil tree, is a subtropical and tropical ornamental plant, which is traditionally used for the treatment of tumors. This plant displays a diverse range of bioactive constituents including the isoeuphorol triterpenoic, taraxasterol, tirucallol , phorbol, ingenane, togliane and diterpenic acid derivatives Controversial in vitro results were found in the literature regarding *E. tirucalli* L properties. Some reports demonstrated its activities as molluscicide, larvicidal, antiviral and cytotoxic against tumoural cells [6].

MATERIALS AND METHODS

Plants material and extract preparation

For the present study the plant was procured from the reliable source of Sangli region, and the identity of the drug was established by morphological study at the Department of Botany, Willingdon College, Sangli. The whole plant of *Euphorbia hirta* Linn were shade dried at 37^{0}C to 40^{o}C and coarsely powdered through mesh 20. The leaves of *Euphorbia nerrifolia* Linn were shade dried and extracted with methanol by using soxhelet apparatus. The powdered plant *Euphorbia hirta* Linn was defatted with petroleum ether 60-80^o C successively extracted with chloroform, ethyl acetate, acetone, ethanol by soxhelet apparatus. The extract was used in the concentration of 100, 200, 300, 400, 500, 600 µg/ml.

Drugs and chemicals

Phosphate Buffer (150mM.pH 7.4), MgCl2 (75mM), NADPH (5mM), aniline (120mM), Sodium carbonate (10%), Phenol (2%) and Na OH (0.2N)

Procedure[7]

The enzyme was induced in rats by oral administration of Phenobarbital (80mg/kg) for 5 days. The animals were sacrificed after an overnight fasting by ether anesthesia and the liver was excised. A 10 % homogenate was prepared in 10mM ice cold tris-HCl buffer (pH 7.4) containing 0.25M sucrose. It was centrifuged at 4^{o}C for 30 min at 9000rpm and used for assay. The reaction mixture contained Phosphate Buffer (150mM.pH 7.4), MgCl2 (05mM), NADPH (0.33 mM), aniline (32mM), enzyme (1-1.5mg protein) and various concentration of extract in a final volume of 1.5 ml. the reaction mixture was incubated for 2 h at 37^{o}C. The reaction was then stopped by the addition of 0.5ml 20% trichoro acetic acid (TCA). The contents were then mixed, centrifuged at 3000 rpm for 10min and treated with 0.5ml of 10% Na2CO3 solution and 1ml of 2% phenol in 0.2N NaOH, mixed and placed in an incubator at 37^{o}C for 30 min. p-aminophenol formed during the enzyme action reacts with phenol in alkaline medium to form a blue coloured product, which was measured at 630nm. The percentage inhibition of aniline hydroxylase was calculated by comparing the absorbance of control and that of drug treated samples.

LETHAL CONCENTRATION (LC_{50}) DETERMINATION

The lethal concentrations of plant extract resulting in 50% mortality of the brine shrimp (LC_{50}) was determined from the 24 h counts and the dose-response data were transformed into a straight line by means of a trend line fit linear regression analysis (MS Excel version 7); the LC_{50} was derived from the best-fit line obtained.

RESULT

Table no. 1 Absorbance of various concentrations of *Euphorbia hirta* L and their % inhibition.

Conc.	Absorbance	Inhibition	% inhibition
100	0.14	0.07	6.67
200	0.13	0.13	13.33
300	0.1	0.33	33.33
400	0.09	0.40	40.00
500	0.07	0.53	53.33
600	0.04	0.73	73.33

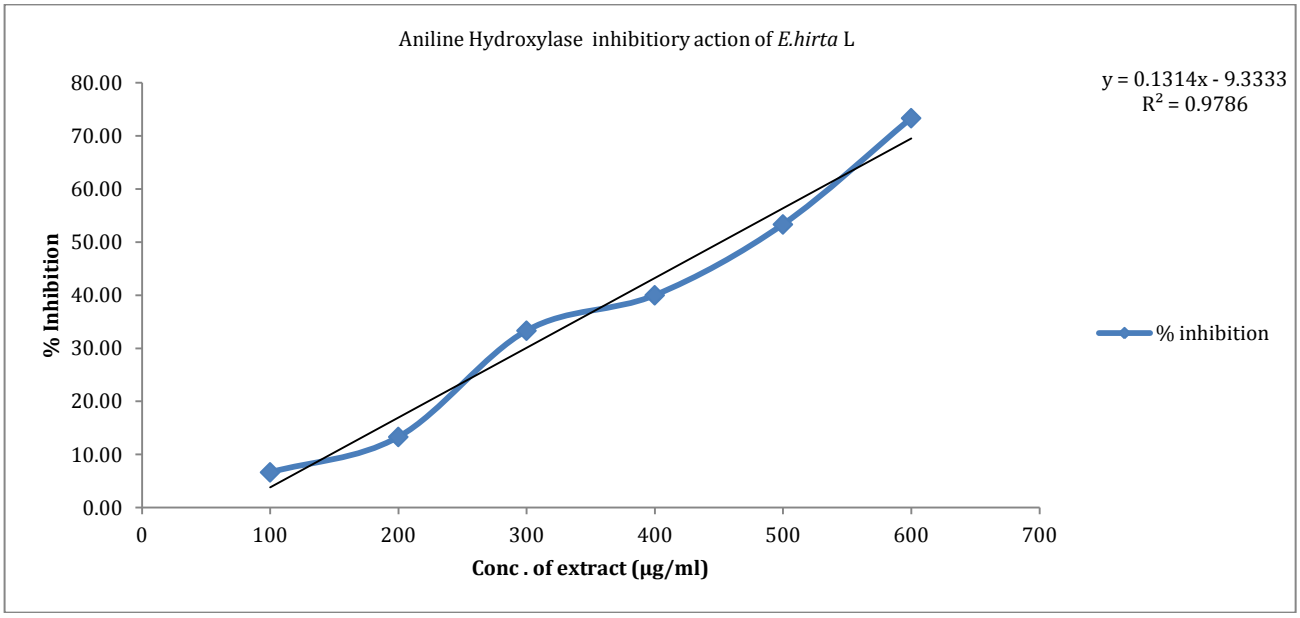
Table no. 2 Absorbance of various concentrations of *Euphorbia neriifolia* Linn and their % inhibition.

Conc.	Absorbance	Inhibition	% inhibition
100	0.14	0.07	6.67
200	0.11	0.27	26.67
300	0.1	0.33	33.33
400	0.09	0.40	40.00
500	0.07	0.53	53.33
600	0.06	0.60	60.00

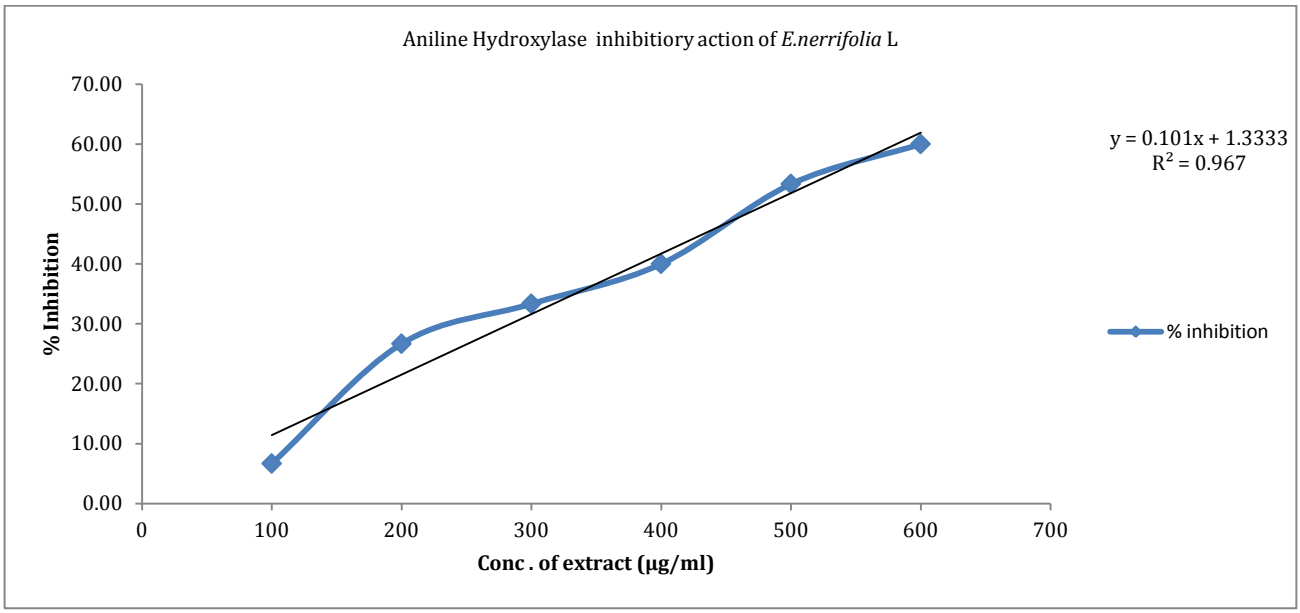
Table no. 3 Absorbance of various concentrations of *Euphorbia tirucalli* Linn and their % inhibition.

Conc.	Absorbance	Inhibition	% inhibition
100	0.11	0.27	26.67
200	0.1	0.33	33.33
300	0.08	0.47	46.67
400	0.07	0.53	53.33
500	0.06	0.60	60.00
600	0.05	0.67	66.67

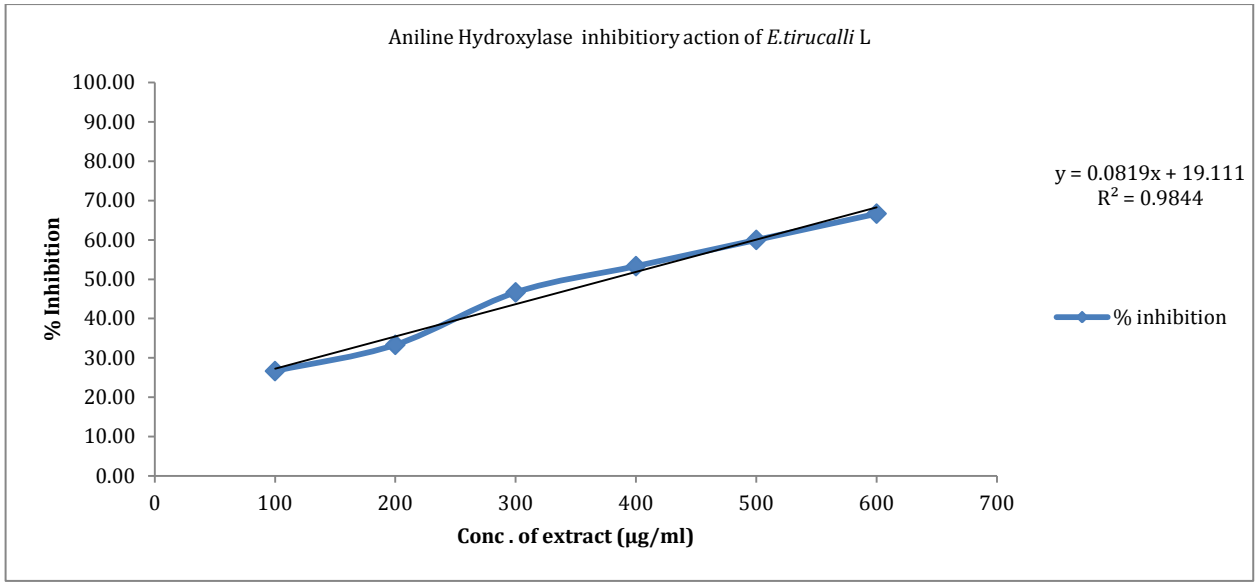
Graph no. 1: LC_{50} or 50 % inhibition of enzyme by *Euphorbia hirta* Linn at various concentrations.



Graph no. 2: LC_{50} or 50 % inhibition of enzyme by *Euphorbia nerrifolia* Linn at various concentrations



Graph no. 3: LC_{50} or 50 % inhibition of enzyme by *Euphorbia tirucalli* Linn at various concentrations



DISCUSSION

E. hirta L extract was found to have significant activity against chemically induced tumour. In a comparison of the activity of two other drugs such as *E. nerrifolia* L and *E. tirucalli* L. *E. hirta* L, was found to be maximally active as seen from the inhibition of liver marker enzymes and proliferative markers [7]. The results indicate that the extract may be more effective in the condition of liver tumours. *E. hirta* L extract showed a dose dependent inhibition of aniline hydroxylase. Concentration required for 50% inhibition of aniline hydroxylase was found to be 310.45 µg/ml and *E. nerrifolia* L and *E. tirucalli* L were showed 481.85 µg/ml and 381.35 µg/ml which were less potent then the E. hirta extracts.

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