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EVALUATION ANTIOXIDANT ACTIVITY OF LEAF EXTRACTS OF *Ficus tinctoria*

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ABSTRACT

The present investigation evaluated the antioxidant activity of leaves of *Ficus tinctoria forester F* stems belonging to the family Moraceae. The methanolic, hydroalcholic, aqueous and n-butanol extracts of *Ficus tinctoria forester F* leaves were prepared and tested for their *in vitro* anti oxidant activity using methods like DPPH antioxidant assay, ABTS scavenging assay and superoxide radical scavenging assay.The n-butanol soluble methanolic extracts has shown IC_{50} value of 81.08µg/ml with superoxide radical scavenging activity while the other extracts had milder activity. The same extract of *Ficus tinctoria* was found to possess good DPPH antioxidant assay shown an IC_{50} value of 64.06µg/ml. This leaf extract has also recorded an IC_{50} value of 46.84µg/ml in ABTS radical scavenging assay.

Keywords: *Ficus tinctoria forester F,* methanolic, hydroalcholic, aqueous and n-butanol extract, *in vitro* antioxidant activity.

INTRODUCTION

Ficus tinctoria is a climbing strangler, forming a tree with prop-roots and grows up to 25m. It has alternately arranged leaves which are oval, glossy dark green above and pale green below with a rounded tip and base [1-5]. Leaves are often asymmetrical with stalks of thickness around 1.5 cm. Fruit is a fig, appearing in leaf axils, usually paired, round. It ripens through orange to red or purple. The fruits are the source of a red dye used in traditional fabric making in parts of Indonesia. *Ficus tinctoria* belongs to family *Moraceae.* Other names of the plant are Dye fig[2], Humped fig, Datir, kaliatthi, Itthi, gudumittemara, Umbar.

An antioxidant is a molecule that inhibits the oxidation of other molecules. Oxidation is a chemical reaction that can produce free radicals, leading to chain reactions that may damage cells [6-8]. Antioxidants are also known as "free radical scavengers." In laboratory and animal studies [9], the presence of increased levels of exogenous antioxidants has been shown to prevent the types of free radical damage that have been associated with cancer development. Therefore, researchers have investigated whether taking dietary antioxidant supplements can help lower the risk of developing or dying from cancer in humans

MATERIALS AND METHODS

Plant Collection, Drying and Pulverization

Leaves of *Ficus tinctoria* were collected from Rampachodavara, Boduluru village, East Godavari district in the month of April. They were authenticated by Dr. K. N. Reddy, Dept. of taxonomy, Laila impex R&D Centre, Vijayawada. The plant materials were deposited in raw drug museum, Laila impex, R&D centre. The voucher no. of *Ficus tinctoria* leaf material was 3319.

The leaves of plant material was air dried under shade at Dept. of taxonomy, Laila Impex R&D Centre and powdered mechanically to coarse or fine powder.

Extraction

The leaf materials of plant was extracted with water, methanol and hydro alcohol (60 % methanol in water) to obtain aqueous, methanolic and hydro alcoholic extracts of various parts of *Ficus tinctoria.*

The leaf methanolic extract of *Ficus tinctoria* was partitioned with chloroform, n- butanol and water. The respective soluble were separated. Codes of extracts were named as below and in the further discussion the extracts were mentioned as their codes.

FTL 01- *Ficus tinctoria* leaf water extract
FTL 02- *Ficus tinctoria* leaf hydroalcoholic extract
FTL 03- *Ficus tinctoria* leaf methanolic extract
FTL 03/01 - *Ficus tinctoria* chloroform solubles of leaf methanolic extract
FTL 03/02- *Ficus tinctoria* n-butanol solubles of leaf

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methanolic extract
FTL 03/03- *Ficus tinctoria* aqueous solubles of leaf methanolic extract

***IN VITRO* ANTIOXIDANT ACTIVITY**

DPPH Radical Scavenging Activity

The DPPH antioxidant assay is based on the ability of DPPH a stable free radical, to decolorize in the presence of antioxidants. The DPPH radical contains an odd electron, which is responsible for the absorbance at 490 nm and also for visible deep purple color. When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized which can be quantitatively measured from the changes in absorbance [10].

DPPH Radical Scavenging activity for the plant extracts was assessed on the basis of the radical-scavenging effect of the stable 1, 1- diphenyl-2-picrylhydrazyl (DPPH) free radical. The concentration of DPPH was kept at 300 µM in methanol. The extracts were dissolved in methanol. 10 µl of each extract solution was allowed to react with 200 µl DPPH at 37°C for 30 min in a 96-well micro liter plate. After incubation, decrease in absorption for each solution was measured at 490 nm using a micro plate reader. Ascorbic acid was used as standard.

ABTS radical scavenging assay

ABTS radical scavenging activity of the extracts was assessed spectroscopically by ABTS cation decolourization assay. The test was based on the relative activity of antioxidants to scavenge the radical cation ABTS and the absorbance were measured at 734 nm.

ABTS radical cations are produced by reacting ABTS (7 mM) and potassium per sulfate (2.45mM) and incubating the mixture at room temperature in the dark for 16 h. The solution thus obtained was further diluted with PBS to give an absorbance of 1.000. Different concentrations of the test sample in 50 ml were added to 950 ml of ABTS working solution to give a final volume of 1 ml. The absorbance was recorded immediately at 734 nm (Auddy et al., 2003). Gallic acid was used as standard. The percent inhibition was calculated from the following equation [11].

% inhibition = [(Absorbance of control – Absorbance of test sample)/Absorbance control] x100.

Superoxide radical scavenging activity

In-vitro super oxide scavenging activity is measured by riboflavin/light/NBT reduction. Reduction of Nitro Blue Tetrazolium is most popular method which is based on generation of super oxide radical by auto-oxidation of riboflavin in presence of light. The super oxide radical reduces NBT to blue colour formation that can be measured at 560 nm.

The scavenging activity towards the super oxide radical (O_2^-) was measured in the terms of inhibition of generation of $O_2^{-\cdot}$ The reaction mixture consists of phosphate buffer (50 mM p^H 7.6), riboflavin (20 µg/0.2 ml), EDTA (12 mM), NBT (0.1mg/3ml). Test compounds of various concentrations of 100-500µg /ml were added to make total volume of 3ml. the absorbance was read at 530 nm before and after illumination under UV lamp for 15 min against a control instead of sample. The percentage inhibition was calculated by using the formula [12].

% inhibition = [(Absorbance of control – Absorbance of test sample)/Absorbance control] x 100.

RESULTS

Ficus tinctoria leaf extracts were evaluated for *in-vitro* antioxidant activity by superoxide radical scavenging, DPPH radical scavenging and ABTS radical scavenging activity. FTL 03/02 was found to be potent for superoxide radical scavenging activity with IC_{50} 81.08µg/ml. In case of DPPH radical scavenging activity FTL03/02 was found to be potent with IC_{50} 64.06µg/ml. For ABTS radical scavenging activity FTL 01, FTL 03, FTL 03/02 and FTL 03/03 showed activity with IC_{50} 33.78µg/ml, 29.65µg/ml, 46.84 µg/ml and 45.07µg/ml. For all the *in-vitro* antioxidant activity results were compared with standards as Gallic acid with IC_{50} value 0.53µg/ml for Superoxide radical scavenging and vitamin-C with IC_{50} values as 4.16µg/ml and 2.47µg/ml for DPPH radical scavenging and ABTS radical scavenging activity.

Table 1. Leaf extraction of *Ficus tinctoria*

Extract	Crude drug	Quantity	Extract obtained	Solvent used
FTL 01	*Ficus tinctoria* leaf	100g	18.80 g	Water
FTL 02	*Ficus tinctoria* leaf	100 g	10.68 g	60% Methanol
FTL 03	*Ficus tinctoria* leaf	100 g	8.03 g	Methanol
FTL 03/01	*Ficus tinctoria* leaf extract	2 g	0.34 g	Chloroform
FTL 03/02	*Ficus tinctoria* leaf extract	2 g	0.67 g	n-butanol
FTL 03/03	*Ficus tinctoria* leaf extract	2 g	0.99 g	Water

***IN-VITRO* ANTIOXIDANT ACTIVITY**

Table 2. Superoxide radical scavenging activity of extracts of *Ficus tinctoria*

S.No	Test compound	Dose (µg/ml)	Percent Inhibition	IC_{50} (µg/ml)
1	FTL 01	25	19.83	>100
		50	29.54	
		100	41.54	
2	FTL 02	25	17.60	>100
		50	25.00	

		100	37.50	
3	FTL 03	25	29.85	>100
		50	34.34	
		100	43.96	
4	FTL 03/01	25	25.57	>100
		50	23.01	
		100	17.99	
5	FTL 03/02	25	24.52	81.08
		50	34.87	
		100	59.00	
6	FTL 03/03	25	9.02	>100
		50	16.26	
		100	25.00	
7	Standard (Gallic acid)	0.25	18.36	0.53
		0.5	44.49	
		0.75	76.69	

Table 3. DPPH radical scavenging activity of *Ficus tinctoria*

S.No	Test compound	Dose (µg/ml)	Percent Inhibition	IC_{50} (µg/ml)
1	FTL 01	25	13.42	>100
		50	21.06	
		100	37.70	
2	FTL 02	25	10.29	>100
		50	14.14	
		100	32.59	
3	FTL 03	25	17.25	>100
		50	24.80	
		100	42.30	
4	FTL 03/01	25	9.78	>100
		50	13.03	
		100	19.34	
5	FTL 03/02	25	19.05	64.06
		50	31.22	
		100	49.14	
6	FTL 03/03	25	11.43	>100
		50	14.01	
		100	23.45	
7	Standard (Vitamin C)	1	17.28	4.16
		2.5	32.88	
		5	58.59	

Table 4. ABTS radical scavenging activity of *Ficus tinctoria*

S.No	Test compound	Dose (µg/ml)	Percent Inhibition	IC_{50} (µg/ml)
1	FTL 01	10	29.31	33.78
		25	41.13	
		50	64.91	
2	FTL 02	25	39.25	83.16
		50	45.28	
		100	52.58	
3	FTL 03	10	30.43	29.65
		25	44.51	
		50	68.06	
4	FTL 03/01	25	16.04	>100
		50	22.47	
		100	32.13	
5	FTL 03/02	10	23.04	46.84
		25	33.33	
		50	52.53	

6	FTL 03/03	10	19.63	45.07
		25	36.05	
		50	53.09	
7	Standard (Vitamin C)	1	23.52	2.47
		2.5	55.16	
		5	97.86	

CONCLUSION

The experiment was done following a simple yet effective method with careful monitoring at each step, be it collection of authentic sample, selection of solvents and extraction procedures, purification of the products and evaluation of their anti oxidant activity using established methods. It was a great learning experience and the results when compared with the standard drugs yielded satisfactory results that enabled us to believe the work was successful and has huge scope for further expansion.

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