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Original Research Article

In Vitro Anticancer Activity of Certain Wild Fruits Collected from Sirumalai Hills, Southern Eastern Ghats, India

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Abstract

The *in vitro* anticancer activity of ethanolic fruit extracts of *Capparis sepiaria* (Capparaceae), *Commiphora berryi* (Burseraceae), *Hugonia mystax* (Linaceae) and *Morinda umbellata* (Rubiaceae) were tested using different cancer cell lines, HeLa (human, epithelial cervical cancer), MCF-7 (human, breast cancer), DLA (ascetic tumour) and L-6 (normal rat muscle cell) by MTT {[3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide]} and SRB (sulforhodamine B) colorimetric assay. The highest cytotoxic activity was recorded in the ethanolic fruit extracts of *Morinda umbellata*. The highest cytotoxicity of 92.85, 78.73, 82.92 and 66.36% was recorded in ethanolic fruit extracts of *Morinda umbellata* against the cell lines, MCF-7, HeLa, DLA and L-6 respectively. The lowest CTC_{50} of 104.2 µg/ml for *Morinda umbellata* fruit extract in MTT assay and 127.8 µg/ml for *Commiphora berryi* extract in SRB assay was recorded against the cells lines, MCF-7 and HeLa respectively. The results clearly indicated that the ethanolic fruit extracts of *Commiphora berryi* and *Morinda umbellata* possess potential *in vitro* anticancer activity against tested cell lines followed by *Hugonia mystax*.

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Introduction

More than 2,500 folkloric Indian medicinal plants have been recorded with a common use of 500 plant species (Jain, 1994). The search for new drugs of natural origin is given importance now-a-days as the medicines for different ailments require attention due to the ineffectiveness and their side effects. Traditional medicinal plants used by local medicinal practitioners are the major sources for drug discovery for different ailments (Karuppusamy et al., 2002). Majority of the medicinal utilities of the plants are known from the ethnic groups of people and their knowledge (Subramanian et al., 2016; Bharali et al., 2016). The medicinal plants in southern Eastern Ghats, Tamil Nadu, India and their biological properties have been little explored (Anandakumar and Karmegam, 2011; Karmegam and Nagaraj, 2014; Nagaraj et al., 2014; Vigneshwari et al., 2014; Karmegam et al., 2015) and requires further scientific evaluation with reference to different parts like leaves, stem, bark, flower, fruits, etc. Wild collected plants and their fruits are reported to possess antioxidant and anticancer capacities worldwide (Wang et al., 2007; Olayiwola et al., 2013; Shad et al.,

2014). In this context, the fruits of certain wild plants, *Capparis sepiaria* L., *Commiphora berryi* (Arn.) Engl., *Hugonia mystax* L. and *Morinda umbellata* L. have been collected for the present study based on our survey in southern Eastern Ghats, for their *in vitro* anticancer activity against four different cell lines.

Materials and methods

Collection and extraction of wild fruits

The fruits of *Capparis sepiaria* L. (Capparaceae), *Commiphora berryi* (Arn.) Engl. (Burseraceae), *Hugonia mystax* L. (Linaceae) and *Morinda umbellata* L. (Rubiaceae) (Fig. 1) were collected from Sirumalai Hills (southern Eastern Ghats), Dindigul district, Tamil Nadu, India and the plant specimens were identified by Dr. S. Karuppusamy, Department of Botany, Madura College, Madurai, Tamil Nadu. The identification was confirmed with local floras. The fruits were collected and shade dried for a week and powdered using ball mill. A fine powder obtained was stored in air tight polythene bags and used for the preparation of extract.

The powdered wild fruit samples (100g for each plant) was taken in a thimble of Soxhlet apparatus and kept the thimble on round bottom flask, and 250ml of each solvent was added to round bottom flask then the condenser was fixed on upper part of the thimble, and finally heated to 50-80°C in a heating mantle. This was done individually for each solvent mentioned above. These steps were carried out for 24 hrs until the extract in the siphon tube became colourless.

After separating the solvents and drying, the final crude extracts were collected and stored individually in air tight containers until use. By using digital electronic balance, 200 mg of each crude extract was carefully taken in a standard measuring flask and 5 ml of ethanol was added to dissolve the extract and 1-2 drops of emulsifier (Triton-X100) was added to completely dissolve the extract. Then it was made up to 200 ml by adding distilled water. This forms the stock solution of 1000 µg/ml (i.e., 1mg/ml), from which different concentrations of test solutions, 500, 250 125, 62.5 µg/ml were prepared and used for *in vitro* anticancer activity.

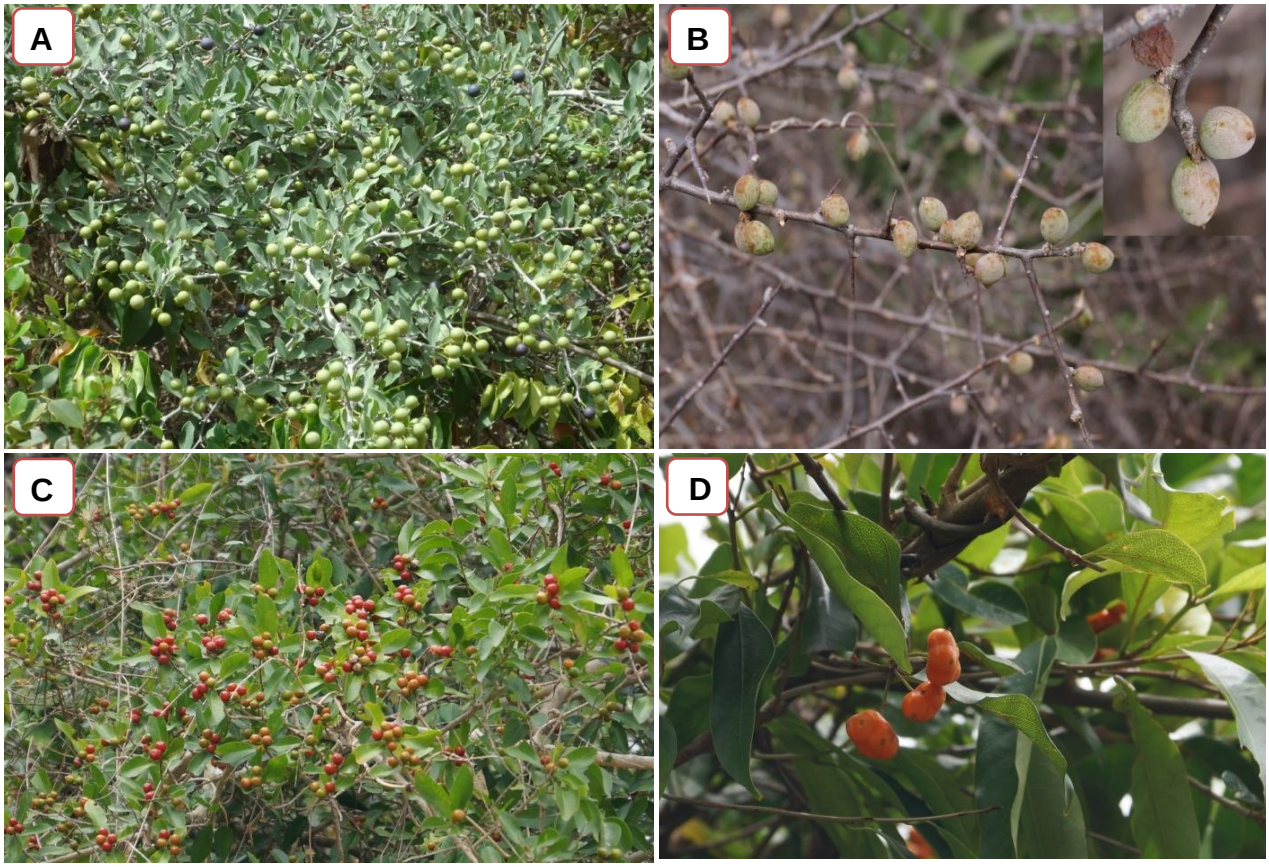

Fig. 1: Fruits of the plants used in the study. **(A)** *Capparis sepiaria*, **(B)** *Commiphora berryi*, **(C)** *Hugonia mystax* and **(D)** *Morinda umbellata*.

Chemicals

MTT assay: 3-(4,5–dimethyl thiazol–2–yl)–5–diphenyl tetrazolium bromide (MTT), fetal bovine serum (FBS), phosphate buffered saline (PBS), Dulbecco's modified Eagle's medium (DMEM) and trypsin were obtained from Sigma Aldrich Co, St Louis, USA. EDTA, glucose and antibiotics from Hi-Media Laboratories Ltd., Mumbai. dimethyl sulfoxide (DMSO) and propanol from E.Merck Ltd., Mumbai, India.

SRB assay: Sulforhodamine B (SRB), FBS, PBS, DMEM and trypsin were obtained from Sigma Aldrich Co, St Louis, USA. EDTA, glucose, trichloroacetic acid (TCA), acetic acid, tris and antibiotics from Hi-Media Laboratories Ltd., Mumbai. DMSO and propanol from E.Merck Ltd., Mumbai, India.

Cell lines and culture medium

HeLa (human, epithelial cervical cancer) and MCF-7 (human, breast cancer), DLA (ascetic tumour) and L-6 (normal rat muscle cell) cell cultures were procured from National Centre for Cell Sciences (NCCS), Pune, India. Stock cells lines were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% inactivated FBS, penicillin (100 IU/ml), streptomycin (100 µg/ml) and amphotericin B (5 µg/ml) in an humidified atmosphere of 5% CO_2 at 37°C until confluent. The cells were dissociated with TPVG solution (0.2% trypsin, 0.02% EDTA, 0.05% glucose in PBS). The stock cultures were grown in 25 cm^2 culture flasks and all experiments were carried out in 96 microtitre plates (Tarsons India Pvt. Ltd., Kolkata, India).

Determination of cell viability

The monolayer cell culture was trypsinized and the cell count was adjusted to 1.0×10^5 cells/ml using medium containing 10% FBS and were used for the determination of cell viability by MTT and SRB assays as described by Francis and Rita (1986) and Philip et al. (1990) respectively. The absorbance was measured using a microplate reader at a wavelength of 540 nm.

The percentage growth inhibition was calculated using the following formula and the results were interpreted using the Duncan's multiple range test. The concentration of test extracts needed to inhibit cell growth by 50% (CTC_{50}) values was generated from the dose-response curves for each cell line.

$$\% \text{ Growth inhibition} = 100 - \frac{\text{Mean OD of individual test group}}{\text{Mean OD of control group}} \times 100$$

Results and discussion

The cytotoxicity of the tested wild fruit extracts showed concentration dependent activity against MCF-7, HeLa, DLA and L-6 cell lines in both MTT and SRB assays (Table 1). In all the test concentrations of the test extracts, higher activity was observed in higher concentrations and low/medium or no activity was found in lower concentrations and significant differences were also noted in cytotoxic activity of each concentration between MTT and SRB and within the cell lines (Table 1). In MTT and SRB assays, highest cytotoxicity level of 92.85% and 87.17% respectively was observed in the ethanolic fruit extracts of *Morinda umbellata* at a concentration of 500 µg/ml against MCF-7 cell lines followed by 85.90% with the extracts of *Commiphora berryi* fruits at a concentration of 500 µg/ml by SRB assay. *Capparis sepiaria* fruit extracts showed moderate cytotoxicity against all cell lines in both MTT and SRB assays and it was the extract showed lowest cytotoxic activity among the wild fruit extracts tested. However, a maximum of at 42.97% and 38.56% cytotoxicity was observed in 500 µg/ml of *Capparis sepiaria* extracts in MTT and SRB assays respectively against DLA cells. The percentage of cytotoxicity of ethanolic fruit extracts of *Commiphora berryi* and *Hugonia mystax* in 500 µg/ml by MTT and SRB assays ranged from 52.43-85.90%. The cytotoxic activity in the lowest concentration of ethanolic fruit extracts used, i.e., 62.5 µg/ml, 33.27% was shown by *Morinda umbellata* against MCF-7 (MTT) followed by 30.17% in *Commiphora berryi* against HeLa (SRB). This shows that the lower concentration of the extracts of *Commiphora berryi* and *Morinda umbellata* fruits in crude form possesses cytotoxicity against cell lines. It has been reported that *Hugonia mystax* methanolic leaf and bark extracts exhibited 72-95% cytotoxicity against HeLa, MCF-7 and A-549 cell lines at 500 µg/ml concentration by MTT assay (Anandakumar and Karmegam, 2011). In the present study, the cytotoxicity shown by ethanolic fruit extracts of *Hugonia mystax* ranged from 52-80% at 500 µg/ml which was slightly lower than that of leaf and bark methanolic extracts as reported earlier. Medicinally the fruits of *Capparis sepiaria* are used for antidote and snakebite (Chopra et al., 1950). Like other species of *Capparis, C. sepiaria* also possess various biological activities (Mishra et al., 2007). However, the cytotoxicity of its fruits is least studied.

Table 1. Cytotoxicity of ethanolic extracts of certain wild fruits collected from Sirumalai hills (southern Eastern Ghats) against different cell lines.

Ethanolic extract used	Test conc. (µg/ml)	Cytotoxicity (%) MCF-7		HeLa		DLA		L-6	
		MTT	SRB	MTT	SRB	MTT	SRB	MTT	SRB
CSF	500	35.14 ^{ab}	28.60 ^{ab}	20.01 ^{b}	32.18 ^{a}	42.97^{a}	38.56 ^{a}	24.11 ^{b}	21.42 ^{b}
	250	21.73 ^{a}	11.92 ^{b}	9.82 ^{a}	20.34 ^{a}	24.15 ^{a}	25.00^{a}	15.86^{b}	13.11^{b}
	125	9.54 ^{a}	3.81 ^{b}	--	13.48	17.06 ^{a}	16.39 ^{a}	10.74 ^{a}	4.60 ^{b}
	62.5	--	--	--	2.70 ^{b}	10.60 ^{a}	10.92 ^{a}	--	--
CBF	500	73. 23 ^{b}	85.90 ^{a}	70.15^{b}	74.00^{b}	63.73 ^{c}	67.37 ^{bc}	59.67 ^{c}	54.79 ^{cd}
	250	55.76 ^{b}	67.45 ^{a}	58.70^{ab}	62.86 ^{a}	54.18 ^{a}	60.90 ^{ab}	32.43 ^{c}	32.36 ^{c}
	125	39.11 ^{b}	45.67 ^{ab}	45.16^{ab}	49.33 ^{a}	39.95 ^{b}	41.05 ^{b}	17.39 ^{c}	14.11 ^{c}
	62.5	24.80 ^{ab}	21.03 ^{b}	29.91 ^{a}	30.17 ^{a}	20.42 ^{b}	25.74 ^{ab}	6.51 ^{c}	9.06 ^{c}
HMF	500	75.60 ^{a}	80.08 ^{a}	68.72 ^{b}	64.11 ^{b}	56.28^{c}	60.99 ^{bc}	52.43 ^{c}	58.03 ^{c}
	250	48.12 ^{b}	59.44 ^{a}	41.14^{bc}	35.91 ^{cd}	33.30 ^{cd}	39.01 ^{c}	29.93 ^{d}	33.83 ^{cd}
	125	26.42 ^{b}	35.23 ^{a}	19.26^{bc}	14.34 ^{c}	12.12 ^{c}	22.34 ^{b}	18.74 ^{bc}	12.16 ^{c}
	62.5	3.57 ^{b}	10.71 ^{a}	--	--	--	7.15 ^{a}	5.91 ^{ab}	--
MUF	500	92.85 ^{a}	87.17 ^{ab}	78.73 ^{b}	83.33 ^{b}	82.92 ^{b}	75.20 ^{b}	66.36 ^{c}	70.25 ^{bc}
	250	80.53 ^{a}	72.93 ^{a}	61.51 ^{b}	50.91 ^{c}	45.17 ^{c}	56.43 ^{b}	40.65 ^{d}	48.91 ^{c}
	125	56.11 ^{a}	40.66 ^{b}	34.95^{bc}	37.01 ^{bc}	21.75 ^{c}	32.58 ^{c}	19.00 ^{c}	23.23 ^{c}
	62.5	33.27 ^{a}	23.00 ^{b}	19.23 ^{b}	21.45 ^{b}	10.62 ^{c}	13.36 ^{c}	8.10 ^{c}	7.80 ^{c}

Ethanolic fruit extracts of *Capparis sepiaria* (CSF), *Commiphora berryi* (CBF), *Hugonia mystax* (HMF) and *Morinda umbellata* (MUF). Date expressed are mean values; Same superscript letters between column values of same concentration did not differ significantly at $p<0.05$ by Duncan's multiple range rest.

The extracts of *Commiphora berryi* alone and in combination with other *Commiphora* species as poly-herbal formulation showed good antioxidant activity and the bark extracts exhibited anti-inflammatory activity (Manivannan et al., 2004; Latha et al., 2012). These activities and the medicinal utility of *Commiphora berryi* is in support of the findings of the present study.

The CTC_{50} values of ethanolic fruit extracts of different wild plants showed a least value of 104.2 µg/ml in *Morinda umbellata* by MTT assay against MCF-7 followed by 127.8 µg/ml (*Commiphora berryi*, SRB assay) against HeLa and 143.7 µg/ml (*Commiphora berryi*, SRB assay) against MCF-7 (Figs. 2 and 3).

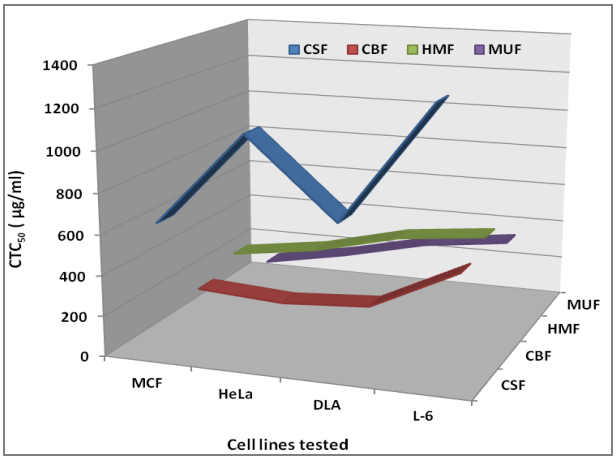

Fig. 2: Cytotoxicity (CTC_{50}) of ethanolic extracts of wild fruits collected from Sirumalai hills (southern Eastern Ghats) against different cell lines by MTT assay.

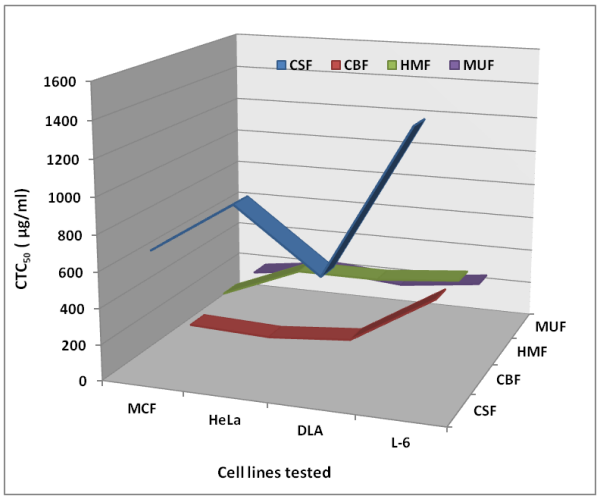

Fig. 3: Cytotoxicity (CTC_{50}) of ethanolic extracts of wild fruits collected from Sirumalai hills (southern Eastern Ghats) against different cell lines by SRB assay.

The CTC_{50} values obtained for the fruit extract of *Capparis sepiaria* showed a range of 644.0-1456.1 µg/ml against the cell lines tested. *Commiphora berryi* fruit extracts showed least CTC_{50} values below 200 µg/ml against all the cell lines, except L-6 cell lines and it ranged between 127.8 µg/ml and 197.5 µg/ml. CTC_{50} value of 188.5 µg/ml was found to be the lowest value against MCF-7 by SRB assay of *Hugonia mystax* fruit extract and the values for other cell lines were above 300 µg/ml. CTC_{50} value obtained for fruit extracts (187-191 µg/ml) is very close the value obtained for leaf and stem bark extracts (Anandakumar and Karmegam,

2011). The present study findings confirm that *Capparis sepiaria*, apart from other biological activities, also exert *in vitro* anticancer activity (Kalpana and Prakash, 2015). Sreenivas et al. (2012) reported that the methanolic bark extract of *Capparis sepiaria* was found to show anticancer activity with an IC_{50} value of 600 µg/ml. Among the four different kinds of wild fruits tested, *Morinda umbellata* is the species which has been least studied for their biological and pharmacological activities (Nagaraj et al., 2014).

The present study clearly shows that the ethanolic extracts of the wild collected fruits of the plants, *Capparis sepiaria*, *Commiphora berryi*, *Hugonia mystax* and *Morinda umbellata* are serving as sources of *in vitro* cytotoxic activities in various capacities against MCF-7, HeLa, DLA and L-6 cell lines. Further studies on phytochemical and pharmacological principles may support the present study findings for utilizing these wild fruits as drugs or for drug formulation.

Conflict of interest statement

Authors declare that they have no conflict of interest.

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