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Research Article

ANTICANCER ACTIVITY OF MERREMIA EMARGINATA (*Burm.F*) AGAINST HUMAN CERVICAL AND BREAST CARCINOMA

Purushoth Prabhu.T*[1], Panneerselvam.P[2], Selvakumari.S[3], Udhumansha Ubaidulla[4], Shantha.A[5]

1. Department of Pharmacognosy, C.L.Baid Metha College of Pharmacy, Thoraipakkam, Chennai, India.
2. Department of Pharmaceutical Chemistry, C.L.Baid Metha College of pharmacy, Thoraipakkam, Chennai, India.
3. Department of Pharmacognosy, Mother Theresa Institute of Health Science, College of Pharmacy, Pondicherry, India.
4. Department of Pharmaceutics, C.L.Baid Metha College of Pharmacy, Thoraipakkam, Chennai, India.
5. Department of Pharmaceutical Analysis, C.L.Baid Metha College of Pharmacy, Thoraipakkam, Chennai, India.

***Corresponding Author: Email**: ubaidnkl@gmail.com

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ABSTRACT

Merremia emarginata Burm. F (Convolvulaceae) is a perennial, much branched herb (creeper). It is found widely distributed all over the India, specially in damp places in upper gangetic plain, Gujarat, Bihar, West Bengal, Western- Ghats, ascending up to 900m in the hills, Goa, Karnataka in India, Ceylon and Tropical Africa. *Merremia emarginata* is also known as *Ipomoea reniformis* chois. It is reported to have many important medicinal properties. In the Indigenous system of Medicine, *Ipomoea reniformis* has been claimed to be useful for cough, headache, neuralgia, rheumatism, diuretic, inflammation, troubles of nose, fever due to enlargement of liver and also for treating cancer. The present study was designed to evaluate the invitro anticancer activity of *Merremia emarginata Burm.F*. The different solvent fraction of whole plant *Merremia emarginata Burm.*F was subjected for MTT assay. The ethylacetate fraction of whole plant was found to be cytotoxic against human cervical carcinoma Hela cell lines and human breast carcinoma MCF cell lines. The IC_{50} value of ethylacetate fraction was 51.57µg/ml against Hela cell lines and 39.6µg/ml against MCF-7 cell lines. Significant results were observed thereby justifying the use of plant in the traditional system of medicine

Keywords: MTT assay, Anticancer activity, *Merremia emarginata*, cervical carcinoma, Breast carcinoma.

INTRODUCTION

Plant derived natural products such as flavonoids, terpenes, alkaloids has been received considerable attention in recent years due to their diverse pharmacological properties, including cytotoxic and cancer chemoprotective effects[1]. Over 50% of the drugs in clinical trails for antitumor activity were isolated from natural source or are related to them[2]. Several plant products have been tested for antitumor activity and some of these, such as vincristine and taxol are now available as drugs of choice[3]. One of the best approaches in the search for antitumour agents from plant resources is the selection of plant based on ethnomedical leads and testing the selected plants efficacy and safety through modern scientific methods[4].

Merremia emarginata Burm. F (Convolvulaceae) is a perennial, much branched herb (creeper). It is found widely

distributed all over the India, specially in damp places in upper gangetic plain, Gujarat, Bihar, West Bengal, Western-Ghats, ascending up to 900m in the hills, Goa, Karnataka in India, Ceylon and Tropical Africa[5,6]. *Merremia emarginata* is also known as *Ipomoea reniformis* chois[7]. It is reported to have many important medicinal properties. In the Indigenous system of Medicine, *Ipomoea reniformis* has been claimed to be useful for cough, headache, neuralgia, rheumatism, diuretic, inflammation, troubles of nose, fever due to enlargement of liver and also in kidney diseases. Powder of leaves is used as a snuff during epileptic seizures, Juice acts as purgative and the root is having diuretic, laxative, and applied in the disease of the eyes and gums[8]. There is no scientific literature for anticancer activity of *Merremia emarginata* and hence the study was designed to investigate the anticancer activity of different solvent fraction of whole plant of *Merremia emarginata* by MTT cell proliferation assay.

MATERIALS AND METHODS

Plant material & Extraction/ Fraction

The plant material were collected in the Tirunelveli region, Tamilnadu, India, in the month of march 2011 and it was botanically identified and authenticated by Prof. Jayaraman, Plant Anatomy Research Centre, Tambaram, Chennai, Tamilnadu, India. The shade dried whole plant of *Merremia emarginata* was coarsely powdered and extracted with ethanol using soxhlet extraction apparatus until exhaustive extraction. The solvent was removed using rotary vacuum evaporator and solvent free fraction were subjected for column chromatography and further fractioned by successive solvents using hexane, chloroform, ethylacetate and methanol solvent were subjected for MTT cell proliferation assay[9].

MTT CELL PROLIFERATION ASSAY

Cell line and culture

The cell line of Hela (Human cervical carcinoma), MCF-7 (human breast carcinoma) were obtained from National Centre for Cell Science, Pune, India. The cells were cultured in a growth medium (DMEM, PH-7.4), supplemented with 10% fetal bovine serum (FBS) and antibiotics, Penicillin (100 units/ml) and streptomycin sulfate (100µg/ml)[10,11].

MTT assay

The cells were seeded into wells of a 96 well microtitre plate (Costar 3599, corning, NY, USA) at 2 x 10^4 cells per well with 100 µl, DMEM growth medium and then incubated for 24 hours at 37°c under 5% CO_2 in a humidified atmosphere. Later, the medium was removed while fresh growth medium containing different test dose at 100, 50, 25, 12.5, 6.25, 3.125µg/ml were added. After 3 days of incubation at 37°c under 5%CO_2, the medium was removed before adding 100µl DMSO to each well and gently shaken[12]. The absorbance was then determined by ELISA reador (Biorad, Mercules, California, USA) at 490nm. Control wells received only the media without the test sample. The conventional anticancer drug, 5-fluorouracil[13], was used as a positive control in this study. The inhibition of cell growth was calculated as a percent anticancer activity using the following formula

$$\text{Cells inhibition} = \frac{\text{Control absorbance} - \text{sample absorbance}}{\text{Control absorbance}} \times 100$$

Statistical analysis

Data were expressed as Means ± standard deviations of three replicate determinations and then analyzed by SPSS v.13 one way analysis of variance (ANOVA) and dancan's new multiple range test were used to determine the differences among the means. P values<0.05 were regarded as significant[14].

RESULTS AND DISCUSSION

The MTT assay is based on the reduction of MTT (3-(4,5-dimethyl thiazolyl)-2,5-diphenyl-tetrazolium bromide) by mitochondrial dehydrogenase to purple formazan product. The different solvent fraction of whole plant *Merremia emarginata* Burm.f were subjected for MTT cell proliferation assay and results are presented in table.1. Among different parts the ethylacetate fraction of *Merremia emarginata* was found to have cytotoxic activity although only fraction with an IC_{50} value lower than 200µg/ml were considered active (Kviecinskie et al.,2008). The other fractions were examined and the IC_{50} value shows higher than 200µg/ml was considered inactive[15]. The photographs (Fig. 1 to Fig. 8) of different fraction of *Merremia emarginata* Burm.F shows the

Different Fraction of Merremia emarginata Burm.F	Cytotoxicity IC_{50} values(µg/ml)	
	Hela	MCF
Hexane (HME)	418.3	434.5
Chloroform(CME)	235.8	211.7
Ethylacetate (EAME)	51.57	39.6
Methanol (MME)	71.5	46.3
5-Fluorouracil	29.6	15.3

Table.1: The IC_{50} values of Merremia emarginata Burm.F against human cervical carcinoma Hela and human breast carcinoma MCF-7 cell lines

apoptosis human cervical carcinoma Hela cell lines and human breast carcinoma MCF-7 cell lines.

CONCLUSION

The MTT assay of different fraction of whole plant of *Merrmia emarginata* Burm.F shows all fraction are having anticancer activity. In that methanol and ethylactate are potent activity in which ethylacetate shows highly potent anticancer activity. The ethylacetate fraction shows 51.57µg/ml and 39.6µg/ml IC_{50} value against Hela and MCF cells respectively. This fraction was able to induce

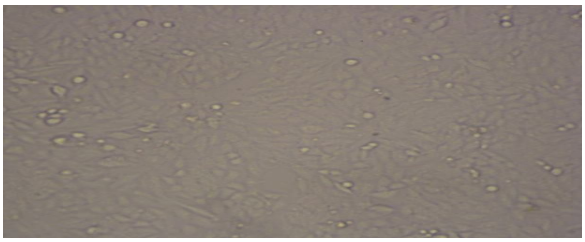

Fig.1 Hela cells treated with MME

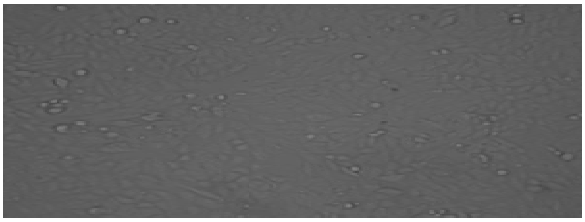

Fig 3 Hela cells treated with EAME

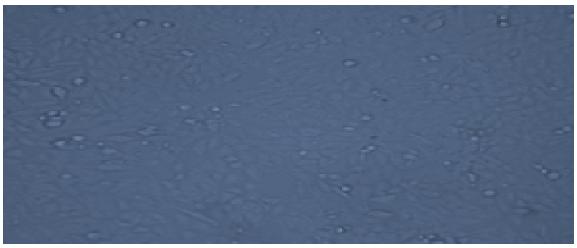

Fig 5 Hela cells treated with HME

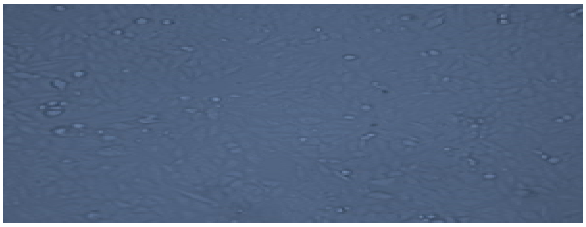

Fig 7 Hela cells treated with CME

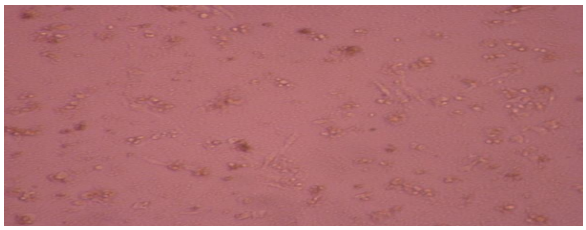

Fig 2 MCF cells treated with MME

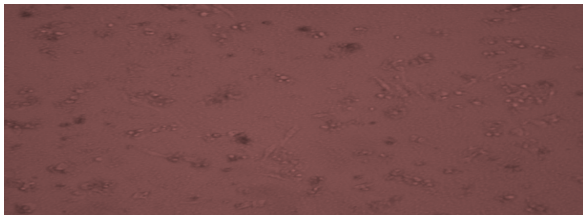

Fig 4 MCF cells treated with EAME

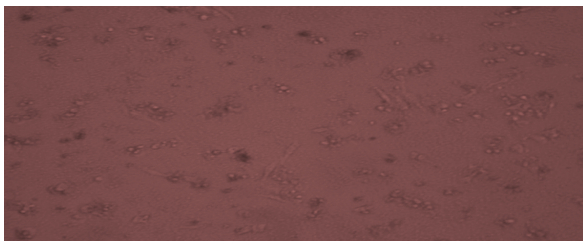

Fig 6 MCF cells treated with HME

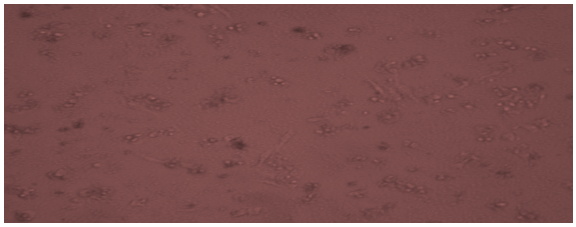

Fig 8 MCF cells treated with CME

apoptosis on human cancer cell lines and its anticancer activity was found to be specific. Further work is required in order to establish the identity of the chemical constituent responsible for anticancer activity. Studies are in progress on our laboratory to elucidate the molecular and cellular mechanism of the ethylacetate fraction invivo which contribute towards the development of potent anticancer drug.

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