INTERNATIONAL JOURNAL OF PHARMACY AND ANALYTICAL RESEARCH

ISSN: 2320-2831

IJPAR |Vol.11 | Issue 2 | Apr - Jun -2022
Journal Home page: www.ijpar.com

Research article *Open Access*

Preliminary invitro anticancer activity studies of various extracts of mesua ferrea l. Seeds

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ABSTRACT

Cancer is a major life threatening disease in both developed and developing countries. The agents which reversed or suppressed tumor progression are termed as anticancer agents. Various studies proved the advantages of natural anticancer agents over synthetic anticancer drugs.Mesua ferrea L. is an ornamental plant and is seen in Asian countries like India, Sreelanka, Andaman Islands, Myanmar, Indo-china, Thailand, Malaysia and Singapore. It is an ornamental tree with reddish brown to grey colored bark. Its leaves are initially red in color and then changes to pale green when it is matured. It is having a white color with floral fragrant flowers which contains numerous golden colored stamens. The flower buds having different medicinal properties against fever, sweats, foul breath, bleeding disorders, small tumors, snake bite etc.The seed oil which was isolated from Mesua ferrea wasused for skin diseases like itching, dandruff, skin eruptions etc. The flowers were used for dysentery, bleeding disorders, rheumatism and iron induced lipid peroxidation. Seed decoctions of this plant were used for gastritis, bronchitis and for curing snake bite. Preliminary screening of various extracts of Mesua Ferrea L. seeds proved the presence of various active principles and also showing antioxidant potentials. On the basis of these preliminary studies invitro anticancer activity screening of four extracts of Mesua ferrea L. seeds were carried out using Trypan blue assay method. Here the cell line used was EAC cell line. Among the four extracts chloroform and petroleum ether extracts showed more prominent cytotoxicity.

Keywords: EAC, PEE, EAE, ELE, CFE

INTRODUCTION

Plants are the major source of natural products having therapeutic potentials. Most of the peoples in developing countries consumes plant derived medicinal agents because of its less side effects compared to synthetic drugs. Many anticancer drugs emerged from natural sources were available in market and being used as a potent remedy for the treatment of cancer.

Cancer is a disease of striking significance in the world today. Cancer is a major cause of morbidity and mortality, with approximately 18.1 million new cases

and 9.6 million cancer related deaths in 2018, affecting populations in all countries and all regions. The increasing cancer burden is due to several factors, including population growth and ageing as well as the changing prevalence of certain causes of cancer linked to social and economic development.

Neoplasm, as defined by Rupert Willis, is an abnormal mass of tissue, the growth of which is uncoordinated with that of normal tissues and that persists in the same excessive manner after the cessation of the stimulus which evoked the change. Cancers are caused by combined genetic and non-genetic changes induced by environmental factors that trigger inappropriate activation or inactivation of specific gene leading to neoplastic transformations or abnormal cell growth.

Although cancer comprises at least 100 different diseases, all cancer cells share one important characteristic: they are abnormal cells in which the process regulating normal cell division are disrupted. That is, cancer develops from changes that cause normal cells to acquire abnormal functions. These changes are often the result of inherited mutations or are induced by environmental factors such as UV light, X rays, chemicals, tobacco products and viruses. All evidence suggests that most cancers are not the result of one single event or factor. Rather, around four to seven events are usually required for a normal cell to evolve through a series of premalignant stages into an invasive cancer.

MesuaferreaL(Calophyllaceae) is a slow growing ever green tree and is widely distributed in tropical countries. It is an ornamental tree with reddish-brown to grey coloured bark. Its leaves are initially red in colour and then changes to pale green when it is matured. It is having a white colour with floral fragrant flowers which contains numerous golden coloured stamens. The flower buds having different medicinal properties against fever, sweats, foul breath, bleeding disorders, small tumours, snake bite etc. It can grow from 30 to 45 meters tall. It is commonly known as Ceylon ironwood, Indian rose, chesenut,or cobra saffron, nagasampige,nagesar, nagchampa.[8] Medicinally, the plant is used in various ailments like rheumatism, as an antidote for snake poison, for bleeding hemorrhoids, cough etc. Various literature reviews pointed out the presence of numerous active principles which are responsible for the above medicinal properties.

MATERIALS AND METHODS

Collection of Plant Material

Plant material was collected from the village areas of Kottayam district of Kerala state. The collected plant materials were identified by Dr. Saju Abraham, Head of the department of Botany, Newman college, Thodupuzha, Idukki district, Kerala.

Extraction

The collected seeds were shade dried and cleaned. And the coarsely powdered seeds were subjected for extraction by using soxhlet extractor for 72 hours with ethanol as solvent. The extract was concentrated by using a rotary evaporator and yielded a semi solid extract. The obtained semisolid extract was subjected to fractional separation using petroleum ether, ethyl acetate and chloroform as solvents. These extracts were used for the preliminary invitro anticancer activity studies.

Invitro Anticancer Activity Studies

The test compound was studied for short term *in vitro* cytotoxicity using Ehrlich Ascites Carcinoma cells (EAC).The tumour cells aspirated from the peritoneal cavity of tumour bearing mice were washed thrice with PBS or normal cell line. Cell viability was determined by trypan blue exclusion method. Viable cells suspension ($1X10^6$ cells in 0.1ml) was added to tubes containing various concentrations of the test compounds and the volume was made up to 1ml using phosphate buffered cell line (PBS). Control tube contained only cell suspension. These assay mixture were incubated for 3 hour at 37^0 C. Further cell suspension was mixed with 0.1ml of 1% trypan blue and kept for 2-3 minutes and loaded on a haemocytometer. Dead cells take up the blue colour of trypan blue while live cells do not take up the dye. The number of stained and unstained cells were counted separately.

$$\% \text{ cytotoxicity} = \frac{\text{No. of dead cells}}{\text{No. of live cells} + \text{No. of dead cells}} \times 100$$

RESULTS AND DISCUSSION

This assay was used to determine the cell viability, where the dead cells get stain and appeared as dark blue in colour. Short term *invitro* cytotoxicity potential of the four extracts were done by trypan blue assay method. The results shown in figures 1,2,3&4 revealed anticancer potency of the four extracts towards Ehrlich Ascites Carcinoma cells (EAC) cell line. The LC50 values of pet.ether, chloroform, ethyl acetate and ethanol found to be 27.28, 24.28, 52.50 and 40.84µg/ml respectively. Petroleum ether extract and chloroform extract showed good IC50 value as compared to other two extracts.

Table 1:

Drug concentration (µg/mL)	% cytotoxicity			
	1 Pet Ether	2 Ethyl Acetate	3 Chloroform	4 Ethanol
1	25.3±1.3	15±1.5	12±1.6	9.08±0.88
3	37.8±2.3	17.5±1.1	17±1.6	12±2.06
5	49.5±1.2	28.1±2.2	31±1.6	18.7±0.97
10	68.2±1.4	43.1±2	41.4±1.6	30.6±1.76
20	96.1±0	46.6±1.7	78±2.1	58.4±1.96
50	100±0	67.4±1.1	100±0	100±0
100	100±0	75.5±1.4	100±0	100±0
200	100±0	95.3±0.9	100±0	100±0
LC50	27.28µg/ml	52.50µg/ml	24.28µg/ml	40.84µg/ml

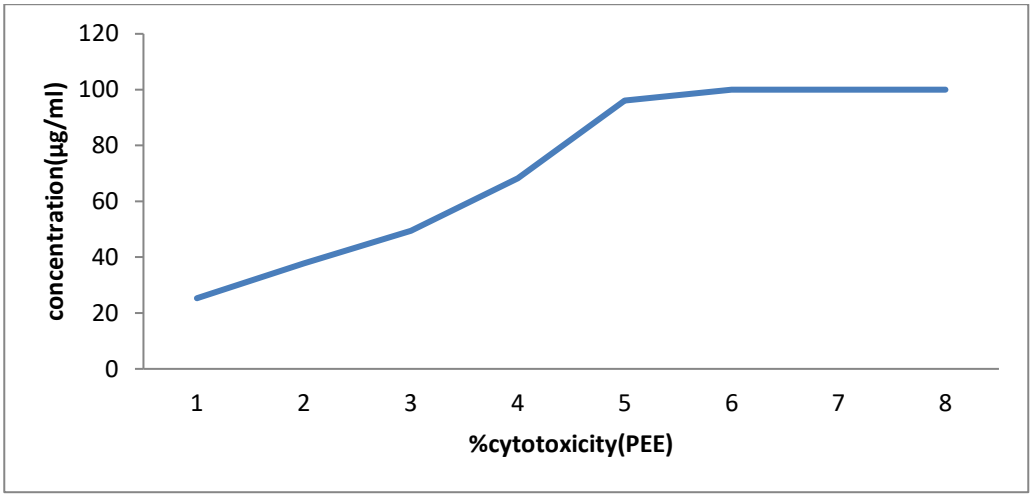

Fig 1: Percentage cytotoxicity of PEE

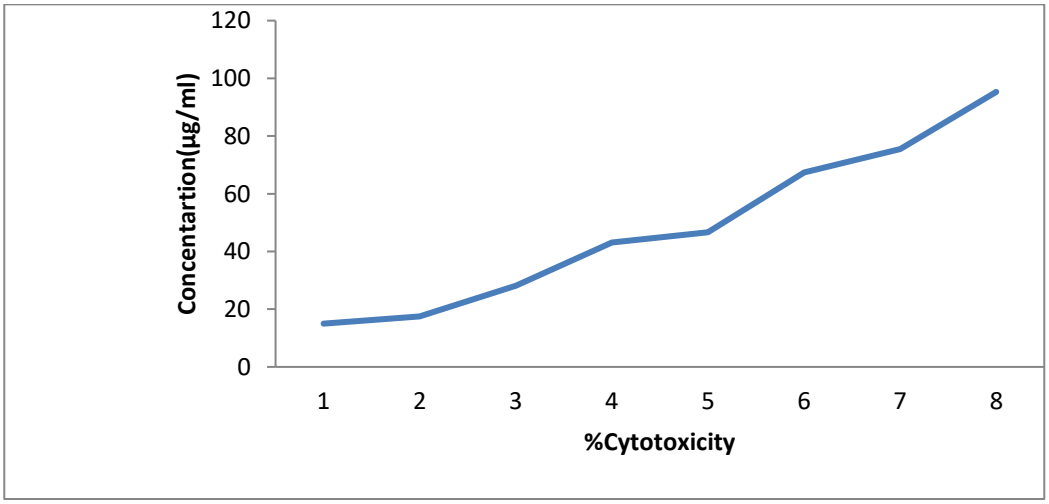

Fig 2: Percentage cytotoxicity of EAE

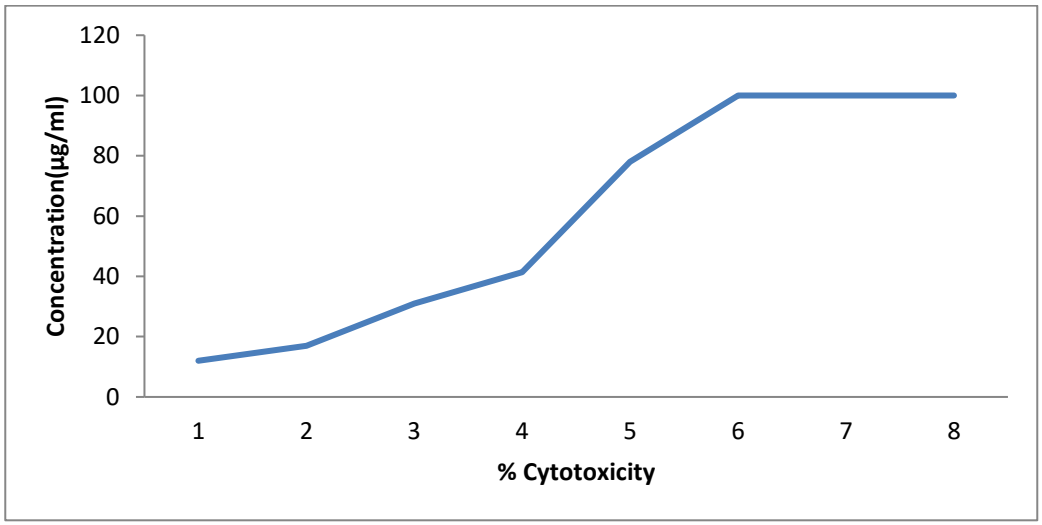

Fig 3: Percentage cytotoxicity of CFE

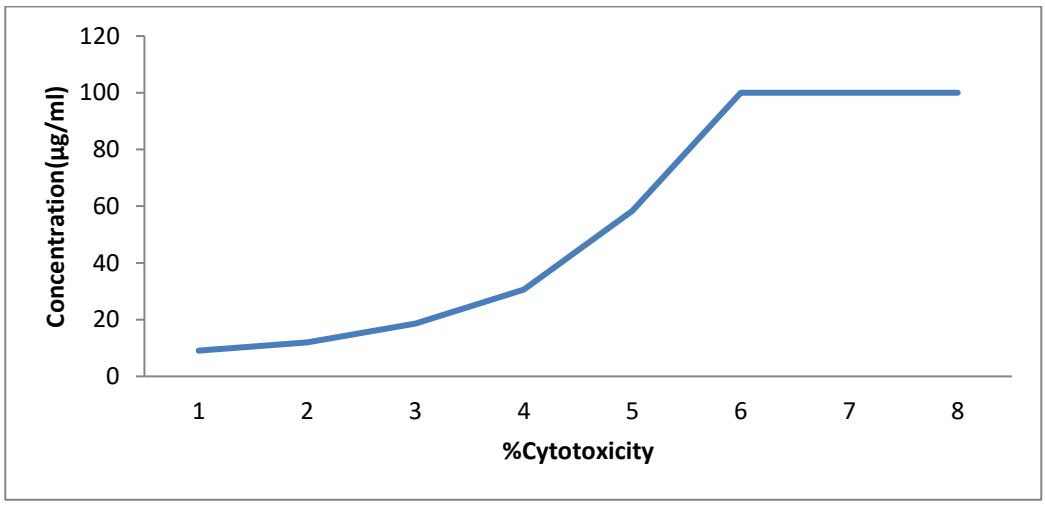

Fig 4: Percentage cytotoxicity of CFE

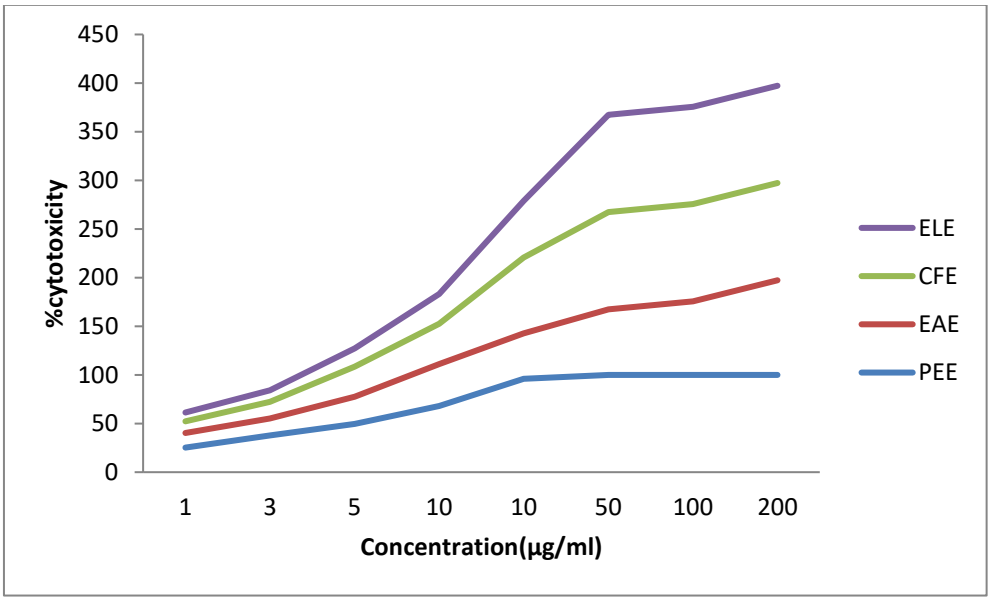

Fig 5: Percentage cytotoxicity shown by PEE, EAE, CFE AND ELE

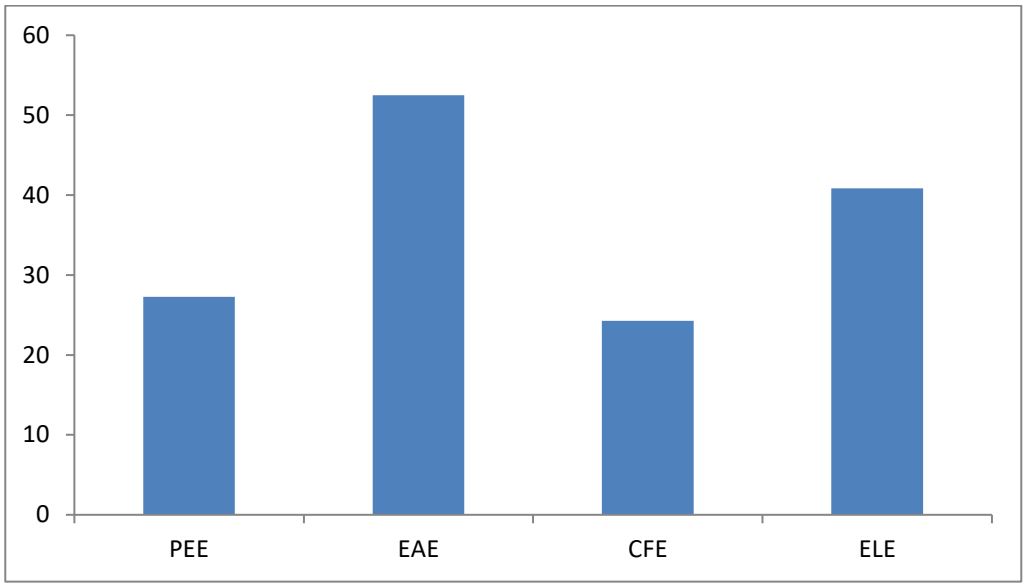

Fig 6: LC 50 Values of test extracts by trypan blue dye exclusion assay

CONCLUSION

The four extracts which were obtained from the seed powder of Mesua ferrea L showed promising invitro anticancer activity by Trypan blue assay method. Out of four extracts Petroleum ether extract and chloroform extract showed good IC50 value compared to other extracts.

ACKNOWLEDGEMENT

We are grateful to Dr. Saju Abraham, HOD, Department of Botany, Newman College, Thodupuzha, Idukki, Kerala for his valuable time for the identification of the plant species.

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