

**EVALUATION OF *INVITRO* ANTICANCER AND ANTI INFLAMMATORY POTENTIAL OF *MITRACARPUS VILLOSUS* (Sw) DC.**

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Corresponding author e-mail:** poonks.che@gmail.com*Received on: 21-03-2017; Revised on: 18-05-2017; Accepted on: 11-06-2017ABSTRACT**

The present study examines the *in vitro* anti inflammatory potential of ethanol and petroleum ether extracts of *Mitracarpus villosus* by HRBC stabilization method. In addition to that *invitro* anti cancer potential of ethanol extract of the *M.villosus* was evaluated by MTT assay. The results revealed that the petroleum ether and ethanol extracts has significant anti inflammatory activities compared to the standard. The petroleum ether extract showed the percentage of lysis of about 25.64 for 20 μ l followed by 36.25, 47.21 for 40 μ l and 60 μ l respectively. The ethanol extract showed % of lysis of 54.24, 66.17 and 78.71 for 20 μ l, 40 μ l and 60 μ l respectively. The ethanol extract showed very good percentage of membrane lysis in all the concentrations employed. The ethanol extract was tested for its anticancer potential against MCF-7-Human breast cancer cell line and HEK- 293-Human embryonic kidney cell line by MTT assay. The ethanol extract showed significant cytotoxic effect on the MCF-7 and HEK-293 cancer cell lines in dose dependant pattern and the IC₅₀ values were determined as 217.6 and 196.8 μ g/ml, respectively. This is the first report of its kind to test the ethanol extract of *M.villosus* for anticancer activity.

Keywords: *Mitracarpus villosus*, anti inflammatory, anti cancer, MTT assay and HRBC membrane**INTRODUCTION**

Cancer is a serious health problem in developing countries due to the excessive environmental pollution, usage of more plastics and pesticides leads to an increase in mortality in the third world countries. Like that inflammations are another health problem faced by human beings. Normally inflammation is the natural defense mechanism against physical trauma, tissue damage, tissue repair and microbiological agents [1]. When tissue cells injured, they release a number of chemicals that indicate inflammatory response. Erythrocyte membrane resembles lysosomal membrane. So the effect of drugs on stabilization of erythrocyte could be extrapolated to the stabilization of lysosomal membrane [2]. In recent years much research is focused on the searching of medicinal plants with anti inflammatory and anticancer activities which may lead to discovery of new crude drugs with low cost and without side effects. *Mitracarpus villosus* (Sw) DC. is

an important plant distributed mainly in tropical and subtropical countries, which belongs to the Rubiaceae family with medicinal properties. Traditionally, it is used to treat sore throat, headaches, toothaches, dyspepsia, hepatic disease, leprosy, eczema, wounds, ulcer and skin diseases [3-5]. Various extracts of the *M.villosus* has various biological activities like antibacterial, antifungal, insecticidal, sedative and anti inflammatory activities [5-12]. In India the leaves of the *M.villosus* is used to cure wound and skin diseases [13]. The phytochemical evaluation of the leaves of this plant contains the presence of alkaloids, tannins, saponins, flavonoids, steroids and terpenoids [5,6,9]. There is no report available on *invitro* anticancer activity of this plant. In this view the present study was focused to evaluate the *invitro* anticancer and anti inflammatory potential of *M.villosus*.

MATERIALS AND METHODS

Plant materials

The plant was collected locally in Pollachi, Tamil Nadu, South India. The specie for this study was identified as *Mitracarpus villosus* (Sw) DC. family Rubiaceae by the Botanical Survey of India, Coimbatore, Tamil Nadu.

Chemicals

Petroleum ether, ethyl acetate, ethanol, sodium chloride dimethyl sulfoxide, 4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT), phosphate buffered saline (PBS), diclofenac, MCF-7-Human breast cancer cell line and HEK 293-Human embryonic kidney cell lines were purchased from NCCS, Pune, was maintained in Dulbecco's modified eagles media (HIMEDIA) supplemented with 10% FBS (Invitrogen) and grown to confluence at 37°C in 5% CO₂ in a humidified atmosphere in a CO₂ incubator. All the chemicals and reagents used were of LR grade.

Extraction process

The plant (1.5 kg) was shade dried, and coarse powdered material was defatted with petroleum ether (6l) by cold maceration for three days with occasional shaking and was subjected to vacuum distillation and was concentrated to yield a greenish residue (19 g). The defatted plant leaves were again extracted with ethanol (4l) using a Soxhlet apparatus. The extract was subjected to vacuum distillation and was concentrated to yield a brownish residue (53 g).

Invitro anticancer activity by MTT assay^[14]

3-[4,5-dimethylthiazol-2-yl]2,5-diphenyltetrazolium bromide (MTT) is a yellow water soluble tetrazolium salt. A mitochondrial enzyme in living cells, succinate-dehydrogenase, cleaves the tetrazolium ring, converting the MTT to an insoluble purple formazan. Therefore, the amount of formazan produced is directly proportional to the number of viable cells. After 48hour of incubation, 15µl of MTT (5mg/ml) in phosphate buffered saline (PBS) was added to each well and incubated at 37 °C for 4h. The medium with MTT was then flicked off and the formed formazan crystals were solubilized in 100µl of DMSO and then measured the absorbance at 570 nm using micro plate reader. The % cell inhibition was determined using the following formula.

$$\% \text{ cell Inhibition} = 100 - \frac{\text{Abs (sample)}}{\text{Abs (control)}} \times 100.$$

Nonlinear regression graph was plotted between % Cell inhibition and Log₁₀ concentration and IC₅₀ was determined using GraphPad Prism software.

HRBC Membrane stabilizing Assay^[15]

The HRBC membrane stabilizing activity assay was

carried out using 10% (v/v) Human erythrocyte suspension, while Diclofenac was used as standard drug. The assay mixtures consisted of 2 ml of hyposaline (0.25% w/v) sodium chloride, 1.0 ml of 0.15 M sodium phosphate buffer, pH 7.4, 0.5 ml of 10% (v/v) human erythrocyte suspension, 1.0 ml of drugs (standard and extracts) and final reaction mixtures were made up to 20-100µl with isosaline.

Drug was omitted in the blood control, while the drug control did not contain the erythrocyte suspension. The reaction mixtures were incubated at 37°C for 30 min and centrifuged at 3000 rpm for 20 min. The absorbance of the supernatant solution was measured spectrophotometrically at 560 nm. Each experiment was carried out in triplicate and the average was taken. The percentage inhibition of haemolysis or membrane stabilization was calculated using the following equation.

$$\% \text{ Inhibition of haemolysis} = 100 \times \frac{(A1 - A2)}{A1}$$

Where: A1 = Absorption of hypotonic buffered saline solution alone

A2 = Absorption of test sample in hypotonic solution

RESULTS AND DISCUSSION

Invitro cytotoxicity by MTT Assay

MTT assay, a simple and reliable technique, which measures cell viability for screening the anticancer activity of plant extracts and synthetic compounds. The viability of cancer cells after incubation with different concentrations of ethanol extract of the leaves of *M.villosus* is depicted in Figure.1. The incubation with different concentration of the extract affected the viability of MCF-7-Human breast cancer cell line and HEK 293-Human embryonic kidney cell line. The ethanol extract showed cytotoxic effect on the MCF-7 and HEK-293 cancer cell lines in the dose dependant pattern and the IC₅₀ values were determined as 217.6 and 196.8 µg/ml, respectively. This is the first report of its kind to test the ethanol extract of *M.villosus* for anticancer activity. The figures 1 & 2 indicated that the ethanol extract of the leaves of *M. villosus* generated a IC₅₀ value of 217.6 µg/ml in MCF-7 model of cancer cell lines and 196.8 µg/ml in the case of HEK-293 model. Relatively low value of IC₅₀ indicated the sample is more cytotoxic and having a good anticancer activity.

Anti-inflammatory activity

The principle involved here is stabilization of human red blood cell membrane by hypotonicity induced membrane lysis. The petroleum ether and ethanol extract of *M. villosus* at a concentration range of 20-100 µl, protect the human erythrocyte membrane against lysis induced by hypotonic solution. The petroleum ether extract showed the percentage of lysis of 25.64 for 20µl followed by, 36.25, 47.21 for 40 µl

and 60 µl respectively. The ethanol extract showed % of lysis of 54.24, 66.17 and 78.71 for 20µl, 40 µl and 60 µl respectively. For higher concentration the methanol and petroleum ether extracts became inactive. The anti-inflammatory activity of both extract was concentration dependent, when the concentration increases the activity also increases. The standard diclofenac showed % of lysis of 65.23, 77.46 and 89.27 at 20, 40 and 60 µl respectively. The ethanol extract showed comparable activity with standard diclofenac, this may be due to the presence of flavonoids, alkaloids triterpenoids and steroids by inhibiting the cox and lox systems [16,17]. Since HRBC membranes are similar to lysosomal membrane components, the prevention of hypotonicity-induced HRBC membrane lysis was taken as measure of anti-inflammatory activity of drugs. The results obtained demonstrated that methanol extract can significantly and dose-dependently inhibits RBC haemolysis. It is well known that the vitality of cells depends on the integrity of their membranes [18]. Exposure of RBC to injurious substances such as hypotonic medium, methyl salicylate or phenyl hydrazine results in the lysis of membrane accompanied by haemolysis and oxidation

of hemoglobin [19]. The haemolytic effect of hypotonic solution is related to excessive accumulation of fluid within the cell resulting in the rupturing of its membrane injury to RBC membrane will render the cell more susceptible to secondary damage through free radical-induce lipid peroxidation. The result revealed that the ethanol extract of *M.villosus* has significant anti inflammatory activity and petroleum ether also showed better activity. Traditionally the plant parts are used to cure inflammation and various types of hepatitis [10]. The present study provides scientific evidence of the important medicinal plant used in folklore.

CONCLUSION

The results of human red blood corpuscle assay of both petroleum ether and ethanol extract of *M.villosus* proved the potent anti inflammatory properties and also evident that ethanol extract has moderate anticancer activity. The isolation of active phytoconstituens is under progress.

Table1. *In vitro* anti-inflammatory activity of petroleum ether and methanol extract of *M.villosus*

Concentration	Petroleum ether extract	Ethanol extract	Diclofenac
	(% of inhibition)		
20µl	25.64	54.24	65.23
40µl	36.25	66.17	77.46
60µl	47.21	78.71	89.27

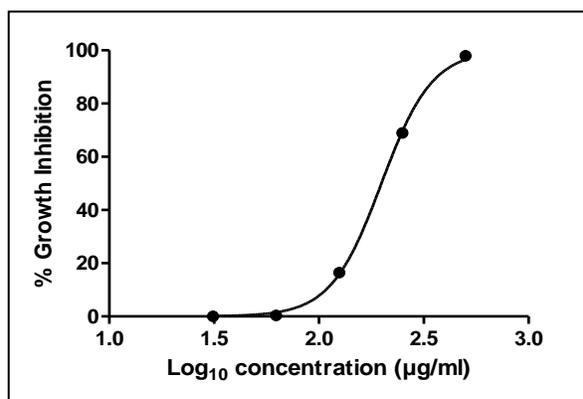
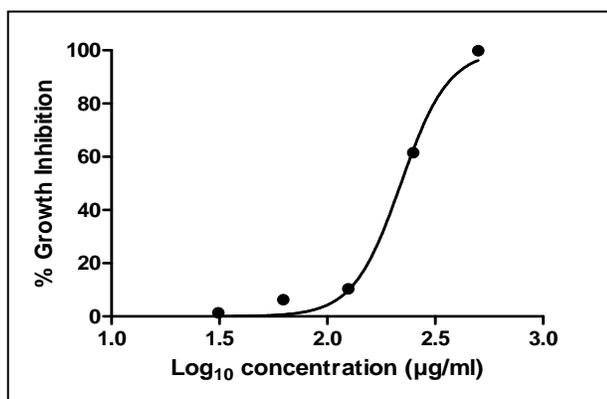


Figure1& 2. *In vitro* cytotoxicity activity of the ethyl acetate extract from *M.villosus* against MCF-7 and HEK-293 cancer cell lines.

Table.2. *In vitro* cytotoxicity activity of ethanol extract of *M.villosus*

Name of the cell lines	IC ₅₀ µg/ml
MCF-7	217.6
HEK-293	196.8

No Conflict of interest**REFERENCES:**

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