IJPSR (2019), Volume 10, Issue 11 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 27 February 2019; received in revised form, 12 June 2019; accepted, 19 July 2019; published 01 November 2019

INHIBITORY EFFECT OF ETHANOL EXTRACT OF *MELASTOMA MALABATHRICUM* LEAVES ON INFLAMMATORY SPLA$_2$ ENZYME AND ITS ABILITY TO REDUCE THE CELL VIABILITY OF PC3 CELL LINE

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Keywords:

Anti-oxidant, *Melastoma malabathricum*, MTT assay, Secretory phospholipase A_2 inhibition, Prostate cancer

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ABSTRACT: *Melastoma malabathricum* is a medicinal plant found throughout Southeast Asian countries. Traditionally *M. malabathricum* was used for treating diseases such as hemorrhoids, cuts and wounds, toothache, and stomachache. Scientific findings also revealed the wide pharmacological actions of various parts of *M. malabathricum* such as anti-nociceptive, anti-inflammatory, wound healing, anti-diarrheal, cytotoxic, and antioxidant activities. The present study evaluated the mechanism of action of the anti-inflammatory activity of *M. malabathricum*. Initially different solvent extracts of *M. malabathricum* were evaluated for total phenolics, total flavonoids, anti-oxidant activity, anti-inflammatory activity (protein denaturation), anti-cancer activity (prostate cancer cell line), and for sPLA2 inhibition. Results showed that ethanolic extract of *Melastoma. malabathricum* leaves showed higher phenolic and flavonoid concentrations, *i.e.* 36.77mg GAE/g dry wt. and 245.5 mg QE/gm of dry weight, respectively. Ethanol extract also exhibited the highest free radical scavenging activity in the DPPH method and phosphomolybdenum assay of which the IC_{50} values were 77.424 µg/ml and 95.159 µg/ml respectively. The anti-inflammatory assay (protein denaturation assay) of leaf extract showed the IC_{50} value of 45.518 µg/ml. Further, the extract exhibited greater sPLA2 inhibition (143.9 µg/ml) in the ELISA method against standard inhibitor thioetheramide-PC (6.73 µg/ml). Finally, the MTT assay for anti-cancer activity showed the IC_{50} value of 630.57 µg/ml against the standard cisplatin on the prostate cancer (PC-3) cell line.

INTRODUCTION: Inflammation is a biological response of vascularized tissues to infections and damaged tissues caused by injury, infection, malignancy, and cellular changes [1]. The function of inflammation is lifesaving. However, if prolonged, it results in undesirable consequences, such as systemic shock, circulatory collapse, arthritis, and local tissue injury in many organs [2-3].

Inflammation is mainly mediated by PLA_2 enzyme that catalyzes the hydrolysis of membrane phospholipids to release arachidonic acid (AA) and lysophospholipids, which are the rate-limiting precursors for the production of pro-inflammatory lipid mediators. The enzymes cyclooxygenase 1/2 (COX 1/2) and lipoxygenase (LOX) catalyze the arachidonic acid into pro-inflammatory mediators

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.10(11).5041-50

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.10(11).5041-50

such as prostaglandins, leukotrienes, and thromboxanes, respectively. The lysophospholipid is converted into platelet activation factors (PAF) by acetyltransferase [4]. An elevated level of $sPLA_2$, which is the type of PLA_2 enzyme is found to be present in various chronic inflammatory diseases including arthritis [5-7], inflammatory bowel disease [8], psoriasis [9], and septic shock [10]. The recent reports revealed the role of $sPLA_2$ in different types of cancers, including breast, colon, pancreas, and prostate cancer [11-12]. Some of the studies have revealed that the eicosanoid pathway is activated in prostate cancer and the cyclooxygenase (COX) and lipoxygenase (LOX) products contribute to the progression of the disease *via* promoting cell proliferation, motility, invasion, and angiogenesis [14-17]. Remarkably, it appears that in some cases $sPLA_2$ plays an oncogenic role and its overexpression is correlated to poor clinical prognosis in prostate cancers [13], but it is apparent that $sPLA_2$ overexpression is associated with aggressive prostate cancer. Prostate cancer is a disease of the elderly with more than three-quarter of the cases occurring in men above 65 years of age. This is globally the second most common cause for cancer and the sixth leading cause of cancer deaths in men worldwide.

The present study deals with the evaluation of role secretory PLA_2 in angiogenesis of prostate cancer because several studies have demonstrated that $sPLA_2$ inhibitors are efficient suppressors of inflammatory processes and thus help in the management of inflammatory phase prostate cancer. Due to the central role of $sPLA_2$ in the inflammatory process and considering the drawbacks and severe side effects exhibited by present anti-inflammatory therapeutic agents, which include the non-steroidal anti-inflammatory drugs (NSAID's) that inhibit either lipoxygenase or cyclooxygenase 1 & 2 enzymes are causing intestinal ulceration, bleeding, and cardiovascular complications. In addition to these problems, COX 1/2 or LOX inhibitors cannot regulate the production of PAF, which continues to cause inflammation [18]. Several endogenous and exogenous agents have been shown to inhibit sPLA2 enzymes [19]. There is renewed pharmacological interest in search of potent and specific sPLA2 inhibitors from diverse sources. In this context, many plant extracts and its constituents are reported for their anti-inflammatory activity through the inhibition of sPLA2. However, effective and specific inhibitors of sPLA2 are still not available to date.

The ancient/traditional medicinal systems primarily used only decoctions of natural sources. Validating crude natural source, identifying, and isolation of specific bioactive principle/s by combinatorial research to target a particular biological pathway has a tremendous scope. Medicinal plants have a long history in both traditional and modern cancer treatments and have been used to treat human diseases for centuries. Thus, traditional medicinal plants can serve as a potential source for developing new drugs and more effective anti-cancer agents for future therapy. The medicinal plant, *M. malabathricum* is commonly known as Doddanekkarika belongs to the genus Melastoma, traditionally used to treat diarrhea, hemorrhoids, cuts and wounds, toothache and stomachache, etc. The *M. malabathricum* showed several pharmacological properties such as anti-bacterial, anti-viral, anti-parasitic, cytotoxicity, anti-coagulant, platelet-activating factor inhibitory, wound healing, anti-ulcer, anti-diarrhoeal, anti-venom, anti-inflammatory, anti-nociceptive, and antipyretic properties at different doses or concentrations [20]. So, presenting the therapeutic and traditional importance of this plant in view, we focused on *M. malabathricum* extracts for sPLA2 inhibition, its anti-oxidant status, and anti-inflammatory activity to prove the plant as a potent anti-cancerous source.

MATERIALS AND METHODS:

Collection and Identification of the Leaves: The *Melastoma malabathricum* leaves were collected from Udupi district, Karnataka, India. The plant was identified and authenticated by Dr. K. Ravi Kumar, Professor, and head, RMR, Foundation of Revitalization of local health Traditions, Bengaluru, Karnataka, India.

Preparation of Extracts and qualitative phytochemical analysis: The plant *M. malabathricum* leaves were washed with distilled water, dried under the shade and ground to a powder form. The powders were packed in Whatman no. 1 filter paper and subjected to Soxhlet extraction with different solvents such as

hexane, chloroform ethanol, methanol, and water. The extracts of organic solvents were concentrated to powder by the flash evaporator, and the water sample was reduced to powder form by lyophilization. The yield was calculated and expressed as % w/w. The phytochemical analyses of different solvent extracts of *M. malabathricum* were performed [21-22].

Estimation of Total Phenol by Folin-Ciocalteu Reagent Method: The total phenolics of the extracts were determined using the Folin-Ciocalteu reagent [23]. For total phenolics determination, Gallic acid was used to make the standard calibration curve. Different concentrations of Gallic acid (10, 20, 40, 60, 80, and 100 µg/ml) and test sample (1mg/ml) made up to 1 ml with ethanol and was mixed with 0.5 ml of Folin-Ciocalteu's phenol reagent (1:1). After 5 min, 1.5 ml of saturated sodium carbonate solution (8% w/v in water) was added to the mixture and the volume was made up to 10ml with distilled water. The reaction was kept in the dark for 2 h, and the absorbance of blue color from different samples was measured at 750 nm. The phenolic content was calculated as Gallic acid equivalents GAE/g of dry plant material based on the standard curve of Gallic acid. All determinations were carried out in triplicates.

Estimation of Total Flavonoid by Aluminium Chloride Colorimetric Method: The aluminum chloride colorimetric method was used for the determination of the total flavonoid content of the extracted sample [23]. For total flavonoid determination, Quercetin was used to make the standard calibration curve. Different concentrations of Quercetin (100, 200, 400, 600, 800, and 1000 µg/ml) and test sample (1 mg/ml) made up to 1 ml with ethanol was mixed with 4 ml distilled water. 0.3 ml of 5% sodium nitrite was added to all tubes and after 5 min, 0.3 ml of 10% aluminum chloride was added. 1M sodium hydroxide was then added to all tubes, and volume was made up to 10 ml with distilled water. After mixing, the solution was incubated for 30 min at room temperature. The absorbance of the reaction mixtures was measured against blank at 510 nm wavelength using the UV-vis spectrophotometer (Labman UV-vis Spectro-photometer). The concentration of total flavonoid content in the test samples were calculated from the calibration plot and expressed as mg Quercetin equivalent (QE)/g of dry plant material. All the determinations were carried out in triplicates.

DPPH (1, 1-diphenyl 2-picrylhyorazyl) Radical Scavenging Assay: In a test tube, 1.5 ml DPPH working solution (0.1 mM) was mixed with 0.5 ml of different concentrations (5, 25, 50, 75 and 100 µg/ml) of plant extract plotted against the standard concentration of ascorbic acid (6, 8, 10, 12, and 14 µg/ml). The samples were incubated for 30minutes in the dark at room temperature. The absorbance was measured at 517 nm (Labman UV visible Spectrophotometer). The percent antioxidant or radical scavenging activity was calculated using the following formula:

$$\% \text{ Antioxidant activity} = [(Ac - As) / Ac] \times 100$$

Where Ac and As is the absorbance of control and sample, respectively. Ascorbic acid was used as standard [24-25].

Phosphomolybdenum Reduction Assay: The total antioxidant capacity of the ethanol extract was evaluated by the phosphomolybdenum reduction assay method [26-27]. The 0.5 ml of various concentrations (10, 15, 20, 25, and 30 µg/ml) of Ascorbic acid and (5, 25, 50, 75, and 100 µg/ml) of extract was combined with 1.5 ml of reagent solution (0.6M sulphuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate) and incubated at 95 °C for 90 min. The absorbance of the reaction mixture was measured at 695 nm using a spectrophotometer.

Anti-Inflammatory Activity by Denaturation assay: A solution of 1% w/v of bovine serum albumin (BSA) was prepared in phosphate-buffered saline (PBS), and pH was adjusted to 7.4 with hydrochloric acid. Stock solutions of 1 mg/ml of the standard (Acetylsalicylic acid) and test extract (Ethanol extract of *M. malabathricum*) were prepared by using suitable solvents. From these stock solutions, different test concentrations, 5-200 µg/ml of test samples were prepared in PBS. 1.6ml of 1% w/v BSA was added to the above test tubes. The control consisted of 1.6 ml of 1% w/v BSA solution with 400 µl PBS. The standard consisted of 100 µg/ml of Ibuprofen in ethanol with 5 ml 0.2% w/v BSA solution. The test tubes were heated at 60 ºC for 10 min and then cooled for 10 min. The absorbance was determined by using a

Spectrophotometer (Labman) at a wavelength of 660 nm. Each experiment was carried out in triplicates, and the mean absorbance was recorded. The percentage of inhibition of precipitation was determined on a percentage basis relative to control using the formula [28-29].

Percentage of inhibition of denaturation = (Abs of control - Abs of extract) × 100 / Abs of control

$sPLA_2$ Inhibitor Screening Assay Kit: This kit was purchased from Cayman chemical kit, and as per the cataloged procedure, the assay was performed [40].

MTT Assay: The cells were trypsinized and aspirated into a 15 ml centrifuge tube. The cell pellet was obtained by centrifugation at 300Xg. The cell count was adjusted using Dulbecco's Modified Eagles (DMEM) medium such that 200 µl of suspension contained approximately 10,000 cells. To each well of the 96 well microtiter plates, 200 µl of the cell suspension was added, and the plate was incubated at 37 °C and 5% CO_2 atmosphere for 24 h. After 2 h, the spent medium was aspirated. 200 µl of different test concentrations (50, 150, 250, 350, and 450 µg/ml from stock) of test drugs were added to the respective wells. The plate was then incubated at 37 °C and 5% CO_2 atmosphere for 24 h. The plate was removed from the incubator, and the drug-containing media was aspirated. 200 µl of medium containing 10% MTT reagent was then added to each well to get a final concentration of 0.5 mg/ml, and the plate was incubated at 37 °C and 5% CO_2 atmosphere for 3 h. The culture medium was removed completely without disturbing the crystals formed. Then 100 µl of solubilization solution Dimethyl sulfoxide (DMSO) was added, and the plate was gently shaken in a gyratory shaker to solubilize the formed formazan.

The absorbance was measured using a microplate reader at a wavelength of 570 nm and also at 630 nm. The percentage growth inhibition was calculated, after subtracting the background and the blank, and concentration of test drug needed to inhibit cell growth by 50% (IC_{50}) was generated from the dose-response curve for the cell line [30-33].

RESULTS:

Phytochemical Analysis: The solvents extracts of *M. malabathricum* plant were prepared, and the phytochemicals were estimated qualitatively and quantitatively. The total phenolics, flavonoids, saponins, terpenoids, and alkaloids were estimated. Ethanol extract of *M. malabathricum* leaves contained the highest phytochemicals, followed by the aqueous extract. Whereas, hexane extract had very fewer photochemical **Table 1**.

TABLE 1: PHYTOCHEMICAL CONSTITUENTS OF *MELASTOMA MALABATHRICUM* LEAVES

Extracts	Phenolics	Alkaloids	Saponins	Terpenoids	Flavonoids
Hexane	+	+	+	-	+
Chloroform	++	+	-	-	+
Methanol	++	+	-	++	-
Ethanol	+++	-	-	+++	+
Aqueous	+++	-	-	+	++

'+': indicates the presence of the respective compound, '-': indicate the absence of the respective compound.

Determination of Total Phenolic Contents in the Plant Extract: The total phenolic content in ethanol extracts of *M. malabathricum* was performed and was expressed in terms of Gallic acid equivalent (calibration curve from Gallic acid showed maximum absorbance at 750 nm wavelengths. (The standard curve equation: y = 0.012x - 0.0379 R^2 = 0.9976). The values obtained for the concentration of total phenols in ethanol extract are expressed as mg of GA/g of extract **Table 2**. The ethanol extract of *M. malabathricum* showed 36.77 mg of GAE/g dry Wt. phenols concentration **Fig. 1**.

Determination of Total Flavonoid Contents in the Plant Extract: The concentration of flavonoids in ethanol extract of *Melastoma malabathricum* Ethanol extract (MMEE) was determined using the spectrophotometric method with aluminum chloride. The content of flavonoids expressed in terms of quercetin equivalent (the standard curve equation: y = 0.0006x - 0.0308 R^2 = 0.9864), mg of QE/g **Table 2**. The quantitative analysis of ethanol extract showed the major presence of flavonoids, *i.e.*, 245.5 mg QE/g dry wt. as depicted in **Fig. 1**.

TABLE 2: TOTAL PHENOLIC AND FLAVONOID CONTENT

Test sample	Phenolic content (mg of Gallic acid equivalent/ g dry material)	Flavonoid content (mg of Quercetin equivalent/ g dry material)
MM ethanolic extract	36.775 mg GAE/g dry wt.	245.5 mg QE/g dry wt.

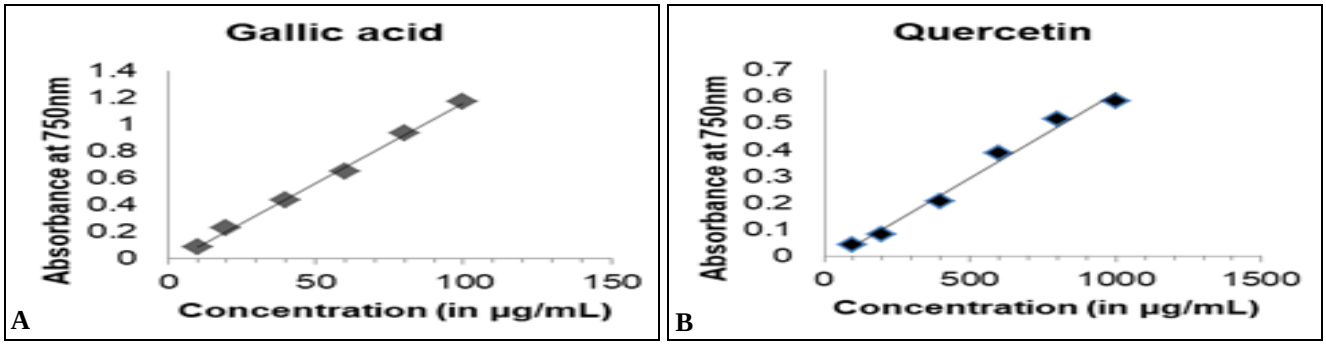

FIG. 1: STANDARD CURVE OF A) GALLIC ACID FOR TOTAL PHENOLICS ESTIMATION B) QUERCETIN FOR TOTAL FLAVONOID ESTIMATION. CONCENTRATIONS OF FLAVONOIDS AND PHENOLICS IN THE PLANT EXTRACT WERE OBTAINED FROM THE STANDARD CURVES

Evaluation of Anti-Oxidant Activity: The investigation of the antioxidant activity of extracts from *M. malabathricum* showed different values in the DPPH method. Among all the extracts, ethanolic extract showed a higher antioxidant activity followed by aqueous extract and methanol extract in a concentration-dependent manner, **Fig. 2a**. Further, only the ethanol extract of *M. malabathricum* was subjected to the anti-oxidant activity by DPPH free radical scavenging assay and phosphomolybdenum reduction assay with different concentrations. By increasing the concentration of *Melastoma malabathricum* Ethanol extract (MMEE), the free radical scavenging activity was shown to get enhanced in a dose-dependent manner, **Fig. 2b**. The IC_{50} value of anti-oxidant activity (DPPH method) of the ethanol extract of *M. malabathricum* was 77.424 µg/ml. Ascorbic acid is a standard whose IC_{50} value is 11.135 µg/ml **Fig. 2c**.

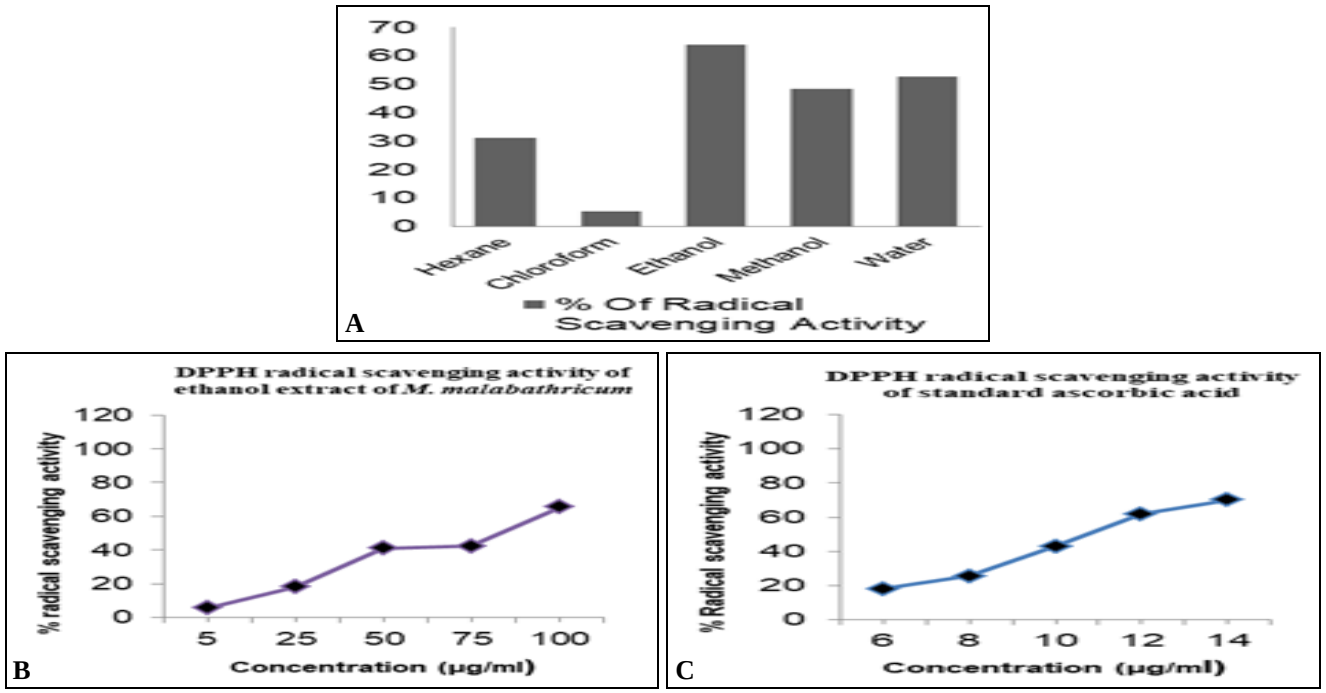

FIG. 2: A) DPPH SCAVENGING ACTIVITY OF *M. MALABATHRICUM* LEAVES EXTRACT, AMONG ALL THE DIFFERENT SOLVENT EXTRACT. ETHANOLIC EXTRACT SHOWED MAXIMUM SCAVENGING ACTIVITY, FOLLOWED BY WATER AND METHANOL EXTRACT AS COMPARED TO OTHER EXTRACT. B) DPPH RADICAL SCAVENGING ACTIVITY OF ETHANOLIC EXTRACT (5, 25, 50, 75, AND 100 µg/ml) OF *M. MALABATHRICUM* LEAVES AGAINST (C) THE STANDARD SCAVENGING ACTIVITY OF ASCORBIC ACID MEASURED AT A FIXED WAVELENGTH OF 517 nm

Similarly, concentration-dependent increase in % relative reducing the power of ethanol extract of *M. malabathricum* in phosphomolybdenum reduction assay was observed, and IC_{50} value of MMEE was 95.159 µg/ml. In comparison with a standard ascorbic acid, IC_{50} value was found to be 13.218 µg/ml **Fig. 3**.

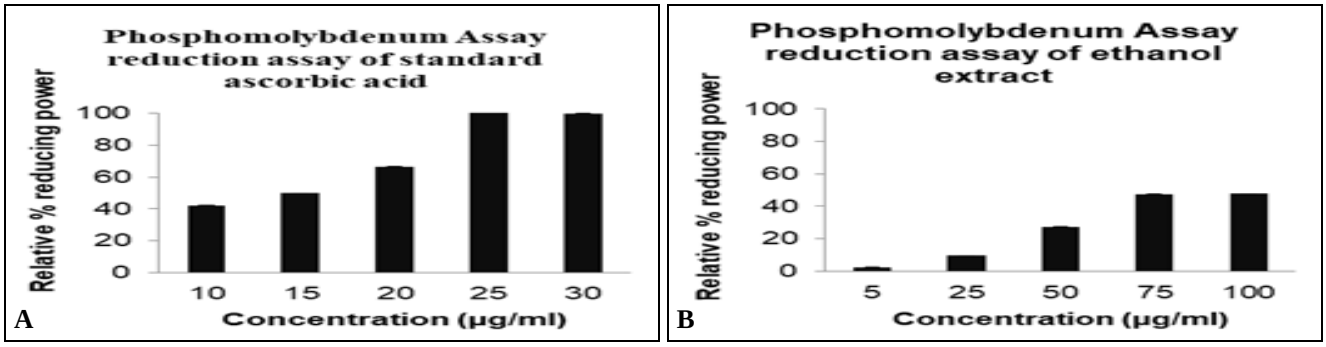

FIG. 3: BAR DIAGRAM REPRESENTING THE PHOSPHOMOLYBDENUM REDUCTION ASSAY OF ETHANOLIC EXTRACT OF *M. MALABATHRICUM* LEAVES (5, 25, 50, 75, AND 100 µg/ml), PLOTTED AGAINST THE STANDARD SCAVENGING ACTIVITY OF ASCORBIC ACID (10, 15, 20, 25, AND 30 µg/ml) MEASURED AT A FIXED WAVELENGTH OF 695 nm

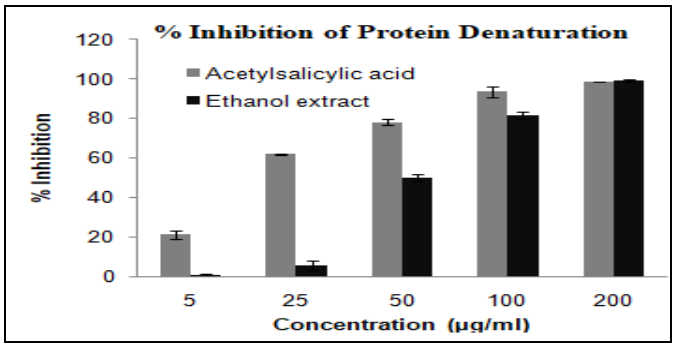

FIG. 4: DIAGRAM REPRESENTING THE COMPARATIVE PERCENTAGE INHIBITION OF PROTEIN DENATURATION OF AN ETHANOLIC EXTRACT OF *M. MALABATHRICUM* LEAVES AND ITS CONCENTRATION WERE (5, 25, 50, 100, AND 200 µg/ml) AND ACETYL SALICYLIC ACID USED AS A CONTROL

Inhibition by Protein Denaturation Assay: As a part of the investigation on the mechanism of the anti-inflammatory activity, the ability of the extract to inhibit protein denaturation was studied. It was effective in inhibiting heat-induced albumin denaturation at different concentrations **Fig. 4**. The protein denaturation was inhibited in a concentration-dependent manner with ethanolic extract at the range 5-200 µg/ml. The IC_{50} value was found to be 45.518 µg/ml. The acetylsalicylic acid, a standard anti-inflammatory drug showed the maximum inhibition at IC_{50} value of 16.137 µg/ml.

$sPLA_2$ Inhibitor Screening Assay: Further, to confirm the anti-inflammatory activity of *M. malabathricum*, the ethanolic extract was subjected to sPLA2 inhibition. Human Type V $sPLA_2$ enzyme was inhibited by ethanol extract of a plant at the concentrations of 5, 10, 50, 100, and 500 µg/ml. The enzyme was found to be inhibited in a concentration-dependent manner. The IC_{50} value of $sPLA_2$ inhibition was found to be 143.942 µg/ml against standard inhibitor thioetheramide-PC 6.73 µg/ml (or 14.804µm) **Fig. 5**.

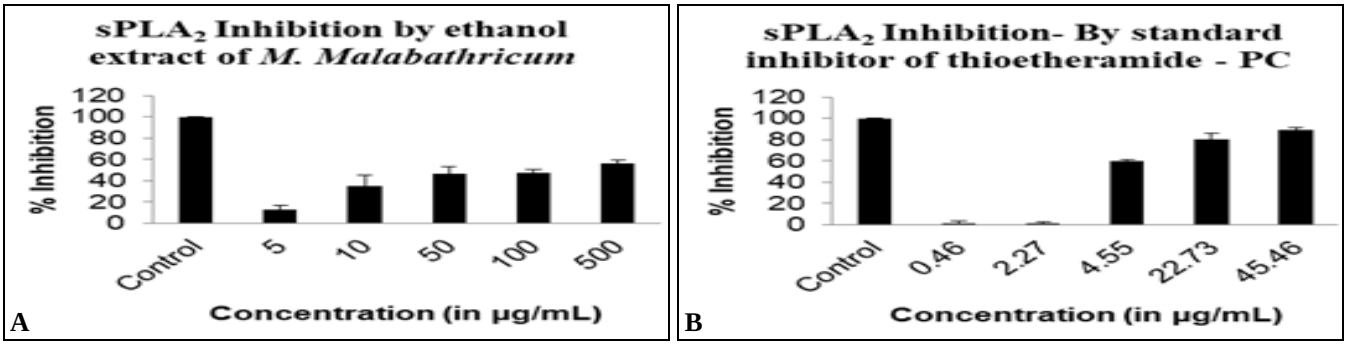

FIG. 5: BAR DIAGRAM REPRESENTING THE ETHANOL EXTRACT OF *M. MALABATHRICUM* LEAVES WITH VARIOUS CONCENTRATIONS (5, 10, 50, 100 AND 500 µg/ml). IT IS PERFORMED AS PER THE $SPLA_2$ INHIBITOR CAYMAN CHEMICAL KIT. CONTROL REPRESENTS THE HUMAN TYPE V $sPLA_2$. (A) FIGURE SHOWS THE PERCENTAGE INHIBITION OF ETHANOLIC EXTRACT OF *M. MALABATHRICUM* LEAVES ON HUMAN TYPE V $SPLA_2$, AND IN THE SIMILAR WAY THE (B) FIGURE SHOWS THE PERCENTAGE INHIBITION OF STANDARD INHIBITOR OF THIOETHERAMIDE –PC ON CONTROL HUMAN TYPE V $sPLA_2$

***In-vitro* Evaluation of Anti-Cancer Activity by MTT Assay:** The MTT assay was carried out on the prostate cancer cell line (PC-3). The percentage viability of the prostate cancer cell line was found to be diminishing with the increase in the concentration of ethanolic extract. The highest decrease in the viability of the cell lines was observed with 450 µg/ml. The viability assay of our extract was compared with the standard cisplatin **Fig. 6**. It was also found that the viability of prostate cancer cell line in the untreated case was 100 percent and the IC_{50} value of the ethanolic extract of *M. malabathricum* for PC-3 cell-line for 24 h treatment was found to be 630.576 µg/ml, against cisplatin (standard) 5.0 µg/ml.

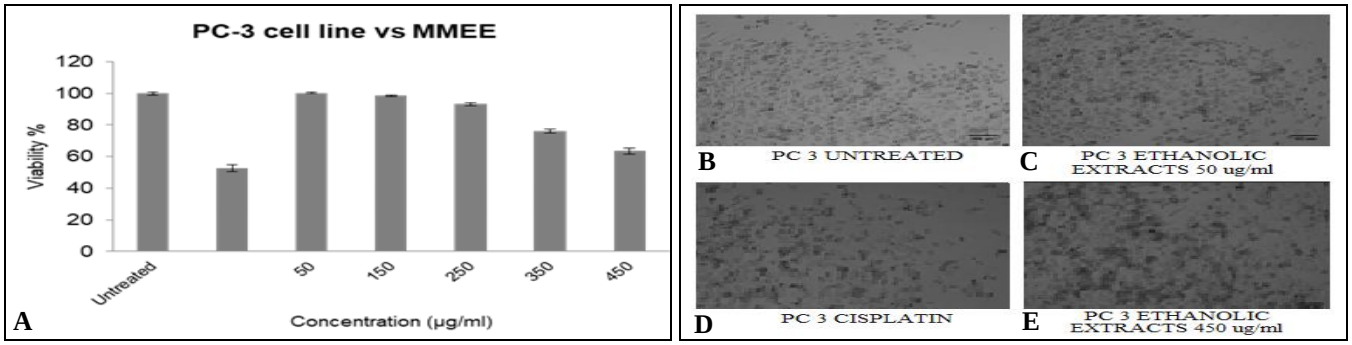

FIG. 6: A) ANTI-CANCER ACTIVITY OF ETHANOLIC EXTRACT OF *M. MALABATHRICUM* AGAINST PC-3 CELL LINE. CISPLATIN WAS USED AS THE STANDARD DRUG; UNTREATED REPRESENTS PROSTATE CANCER CELL LINE. THE PROSTATE CANCER CELL LINE WAS TREATED WITH AN INCREASING CONCENTRATION OF MMEE (50, 150, 250, 350, AND 450 µg/ml). MTT ASSAY ON PROSTATE CANCER CELL LINE B) UNTREATED PC-3 CELL LINE C) TREATED WITH ETHANOLIC EXTRACT OF *M. MALABATHRICUM* ON PC-3 CELL LINE 50 µg/ml d) PC-3 CELL LINE TREATED WITH CISPLATIN STANDARD DRUG E) TREATED WITH ETHANOLIC EXTRACT OF *M. MALABATHRICUM* ON PC-3 CELL LINE 450 µg/ml

DISCUSSION: The chemical substances derived from plants are of potential interest for therapeutic intervention in inflammatory diseases. They act either by inhibiting the pro-inflammatory enzymes (PLA_2, COX, and LOX) or by inhibition of cytokines (IL-1β, TNF-α) release and inhibition of mast cell degranulation that are known contributors to chronic inflammatory disorders [34]. Also, arachidonic acid is metabolized into several molecules by various enzymes, most of which induce cancer cell growth and proliferation [35]. The sPLA2 enzymes catalyze the rate-limiting step in the production of pro-inflammatory eicosanoids and free radicals. Hence, PLA2 inhibition is legitimate in the neutralization of inflammation.

The PLA_2 inhibitors such as flavonoids, retinoids, and terpenoids are anti-oxidants known to act as anti-inflammatory compounds by scavenging the free radicals [37, 38]. Antioxidants also prevent lipid peroxidation caused by reactive oxygen species (ROS) and thus prevent the formation of lipid peroxides, which may, in turn, activate PLA_2 enzyme. ROS also activates PLA_2 and therefore, scavenging of these ROS by anti-oxidants prevents PLA_2 mediated activation of the arachidonic acid cascade [39]. Therefore, a single molecule having both potencies in PLA_2 inhibition and anti-oxidant activity could serve as a better anti-inflammatory molecule.

Screening experiments provide a platform for pharmacological studies that may open the possibility of finding new, clinically effective anti-inflammatory compounds. Phytochemical analysis of different solvent extracts of *Melastoma malabathricum* leaves showed the different spectrum of phytochemical constituents. The ethanol extract of leaves of *M. malabathricum* showed higher phenolic and flavonoid content.

Anti-oxidant activity of different solvent extracts of *M. malabathricum* leaves was checked by DPPH radicals scavenging assay. Among all the extracts, ethanolic extract showed better activity in the DPPH method. Further, ethanolic extract of *M. malabathricum* showed higher hydroxyl radicals scavenging activity and increase in phosphomolybdenum reduction activity in the concentration-dependent manner **Fig. 3**. These results suggest the correlation between the phytochemical contents and antioxidant activities.

Phenylbutazone, salicylic acid, flufenamic acid, *etc*., are some of the anti-inflammatory drugs which have shown dose-dependent ability to thermally induced protein denaturation [36]. Evaluation of the anti-inflammatory potential of ethanolic extract of *M. malabathricum* was carried out by the protein denaturation method. The result demonstrates that the ethanolic extracts of *M. malabathricum* effectively inhibited the denaturation of BSA *in-vitro*. It can, therefore, be concluded that *M. malabathricum* extract possesses significant anti-inflammatory activity.

To evaluate the mechanism of action of anti-inflammatory activity of *M. malabathricum* extract, $sPLA_2$, a key enzyme of inflammation was subjected to inhibition by the extract. The potent active principles from *M. malabathricum* leaves might have exhibited a higher degree of sPLA2 inhibitory action.

Further, the work extended to cell viability assay to check whether the plant is presenting any role in cell viability. The evaluation of the anticancer activity of plant extracts is essential for safe treatment. It enables identification of the intrinsic toxicity of the plant and the effects of an acute overdose [41-42]. *In-vitro* cytotoxicity test using (PC-3) prostate cancer cell lines was performed to screen potentially toxic compounds that affect basic cellular functions and morphology. The MTT cell viability test was conducted to study the effect of *M. malabathricum* extract on the growth of prostate cancer cell lines (PC-3). The ethanol extract significantly inhibited the growth of PC-3 cell line. The result of MTT assay revealed that the ethanol extract of *M. malabathricum* leaves decreased the percent viability of the cell line in a dose-dependent manner. However, the standard cisplatin drug showed significant inhibition on the cancer cell lines. The results revealed morphological changes and shrinkage of cells leading to cell death induced by the extracts in the prostate cancer cell lines **Fig. 6**.

CONCLUSION: The present findings of phytochemical screening of the *M. malabathricum* plant confirmed the presence of several bioactive compounds like phenolics, flavonoids, and terpenoids, which could be responsible for the versatile medicinal properties. The ethanol extract of *M. malabathricum* exhibited a very good anti-oxidant and protein denaturation activity. Also, *M. malabathricum* potentially inhibited $sPLA_2$ type V and showed anti-cancer activity on the PC-3 cell line. Therefore, our study validates the claim regarding this plant for being a potent anti-oxidant and for possessing anti-inflammatory and anti-cancerous activities. However, several important questions remain vulnerable, and additional investigations are necessary to understand the mechanism underlying the effects of the extract and their active compound/s responsible for the anti-inflammatory, anticancer activities.

ACKNOWLEDGEMENT: The authors are thankful to UGC NFSC fellowship, Cell Kraft Biotech Pvt. Ltd. Bangalore, for providing the required facility to carry out cell line work. Also, I extend my sincere thanks to Dr. Nagendra Sastry Yarla, Faculty member, Novel Global Education Foundation Sydney, Fasil Ali, and Sanjana Nayak, Department of studies and Research in Biochemistry, Jnana Kaveri Post Graduate Centre, Mangalore University, Chikka Aluvara for providing support to carry out the work.

FINANCIAL SUPPORT AND SPONSORSHIP: This research did not receive any specific grant from funding agencies in public, commercial, or not for profit sectors.

CONFLICT OF INTEREST: The authors have declared no conflict of interest.

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How to cite this article:
Sophiya P, Lohith NS, Giresha AS, Narayanappa M, Meti RS, Sathisha AD and Dharmappa KK: Inhibitory effect of ethanol extract of melastoma malabathricum leaves on inflammatory sPLA2 enzyme and its ability to reduce the cell viability of PC3 cell line. Int J Pharm Sci & Res 2019; 10(11): 5041-50. doi: 10.13040/IJPSR.0975-8232.10(11).5041-50.