International Journal of Modern Science and Technology *Vol. 1, No. 8, November 2016. Page 283-287.*
http://www.ijmst.co/ ***ISSN: 2456-0235.***

Research Article

Wound Healing Ethano-pharmacological Potential of *Anisomeles malabarica*

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Abstract

Chemotaxonomic examination or ethanobotonical information was used to consider the therapeutic activity of a plant for a particular disease. A loss of cellular and functional continuity of living tissue termed as wound. The infection causing microbes at the wounded site develop resistance against the targeted antibiotics used in controlling microbes and curing wounds. Extraction of novel biologically active compounds from plant species is thereby necessary to cure human ailments especially controlling microbes and curing wounds. This can be achieved by topically applying the compounds with free radical scavenging properties. On considering the above importance, the present study was undertaken to evaluate the anti-oxidant and wound healing activity of medicinal plant *Anisomeles malabarica* . The leaf extracts of *Anisomeles malabarica* exhibited antioxidant activity which was assayed using DPPH free radical scavenging assay. The leaf extracts were subjected to Cytotoxicity assay against Vero cell lines. *Anisomeles malabarica* extracts showed better activity than and was selected for the wound healing assay.

Keywords: Antioxidant; *Anisomeles malabarica*; Cytotoxicity; Wound healing.

Introduction

A wound is a distinct break up in the epithelial integrity of the skin. A wound interrupts skin continuity and integrity. In general, a wound may be the result of trauma or by minor or major abrasions caused in skin due to burns and surgical procedures. In the ancient scriptures of Ayurveda, one of the Indian traditional systems of medicine, the area of wounds and wound healing include clinical details and plants efficient for treatment of wounds, are discussed under the head, "Vranaropaka" [1]. Maharshi Agnivesha was the first To examine the wounds as a medical problem in Agnivesha tantra (later known as Charaka Samhita) as "Vrana" [2] According to Ayurveda, Vrana is the discontinuation of the lining membrane which, after healing, leaves a scar for life, directly similar to the modern definition of wound. In fact the Charak Samhitahas mentioned the use of many different herbs, leaves, oils, stalk of plants, etc. anticipated for the treatment of different types of wounds and inflammation [1,3] have noted that about 70% of the therapeutic Ayurvedic drugs used for wound healing are of plant origin, 20% of mineral origin, and the remaining 10% arise from animal products [4].

In the present investigation the *Anisomeles malabarica* was used for wound healing and antioxidant property. *Anisomeles Malabarica* belongs to family of *Lamiaceae (Labiatae)*, it is an highly aromatic plant, the plant leaf extracts were examined for wound healing and antioxidant property and anticancer activity was carried out on Vero cell lines, therefore the present investigation is part of the continuing program related to phytochemical analysis..

Materials and methods

Plant sample collection

Fresh leaves of *Anisomeles malabarica* were collected from the fields located in Kelambakkam forest, Chennai.

Received: 14.11.2016; Received after Revision: 15.11.2016; Accepted: 15.11.2016; Published: 24.11.2016

Preparation of plant extracts

The leaves were carefully washed with tap water, rinsed with distilled water, and air dried for 1 hour. Then leaves are separated & dried in room temperature for one week. Then they were ground into powder and stored in room temperature. The Direct extraction with chloroform, ethyl acetate and methanol following the method of [5] was used as an extraction method. In this method, finely ground plant material was extracted with chloroform, ethyl acetate and methanol in the ratio of 1:10 in conical flask in shaking condition for overnight. The extract was filtered through the Whatmann No. 1 filter paper in a separate container. The process was repeated 3 times and the same plant material but using fresh solvent. The solvent was removed by placing the extracts in distillation unit in the respective temperature. The extracted residues were weighed and re-dissolved in different solvents to yield 10mg/mL solutions ready for further analysis.

Antioxidant activity assays

DPPH assay

The Radical Scavenging Activity (RSA) of different extracts was determined by using DPPH assay according to [6] with small modification. The decrease of the absorption at 517nm of the DPPH solution after the addition of the antioxidant was measured in a cuvette containing 2960µl of 0.1m Methanolic DPPH solution mixed with 40µl of 20 to 200 µg/mL of plant extract and vortexed thoroughly, The setup was left at dark in room temperature and the absorption was monitored after 20 minutes. Ascorbic acid and Butylated hydroxytoluene (BHT) were used as references. The ability of the plant extract to scavenge DPPH radical was calculated by using equation 1.

$$\% \text{ RSA} = \frac{\text{Abs. control} - \text{Abs. sample} * 100}{\text{Abs. control}} \quad \text{..(1)}$$

Metal chelating activity

The chelating of ferrous ions by methanol extract of *A. malabarica* was estimated by the method of Denis et al [7]. Briefly the extract samples (250µl) were added to a solution of 2mmol/L FeCl2 (0.05mL). The reaction was initiated by the addition of 5mmol/L ferrozine (0.2mL) and the mixture was shaken vigorously and left standing at room temperature for 10 min. Absorbance of the solution was then measured spectrophotometrically at 562 nm. The chelating activity of the extracts was evaluated using EDTA as standard. The results were expressed as mg EDTA equivalent/g extract.

Quantitative analysis of phytochemicals

GC/MS analysis

The methanol extracts were analyzed using the Thermo Scientific GC-MS (SHIMADZU QP2010) gas chromatograph Software: GCMS solution ver.2.53 [8]. It was fitted with a split less injector and connected to an MS Polaris Q-Quadrupole Ion Trap (Thermo Electron) fused silica column VB5 (5% phenyl, 95% methylpolyxiloxane, 30 m with 0.25 mm i.d. film thickness 0.25 µm) (J & W Scientific Fisons, Folsom, CA). The injector and interface were operated at 250 and 300°C, respectively. The oven temperature was programmed as follows: 50°C was raised to 250°C (4°C/min) and held for 3 min. Helium was the carrier gas at 1 ml/min. The sample (1µl) was injected in the split mode (1:20). MS conditions were as follows: ionization voltage EI of -70 eV, mass range 10 - 350 amu. The Methanol extracts components were identified by comparing their relative retention times and mass spectra with those of authentic samples (analytical standards from data base).

Wound healing assay

The Cytotoxicity of samples on Vero was determined by the MTT assay. Cells (1 × 105/well) were plated in 1ml of medium/well in 24-well plates (Costar Corning, Rochester, NY). After 48 hours incubation the cell reaches the confluence. Then, cells were incubated in the presence of various concentrations of the samples in 0.1% DMSO for 48h at 37°C. After removal of the sample solution and washing with phosphate buffered saline (pH 7.4), 200µl/well (5mg/ml) of 0.5% 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl--tetrazolium bromide cells(MTT) phosphate- buffered saline solution was added. After 4h incubation, 0.04M HCl/ isopropanol were added. Viable cells were determined by the absorbance at 570nm [9]. Measurements were performed and the concentration required for a 50% inhibition of viability (IC50) was determined graphically. The absorbance at 570 nm was measured with a UV-

Spectrophotometer using wells without sample containing cells as blanks. The effect of the samples on the proliferation of Vero cells was expressed as the % cell viability, using equation 2.

% cell viability = (A570 of treated cells / A570 of control cells) × 100% ...(2)

Results

DPPH assay of Anisomelos malabarica

From the dose dependent response curve of DPPH radical scavenging activity of different leaf extracts of (Figure 1), *A. malabarica* was observed that the Methanol extract had higher scavenging activity than ethyl acetate and chloroform (Figure 1). At a concentration of 40µg/mL, the scavenging activity of Methanol extract reached 89%, which was comparable to that of standard chemical. The Methanol leaf extract of *A. Malabarica* showed excellent antioxidant and free radical scavenging activity. In considering this, the Methanol leaf extract was chosen for further study.

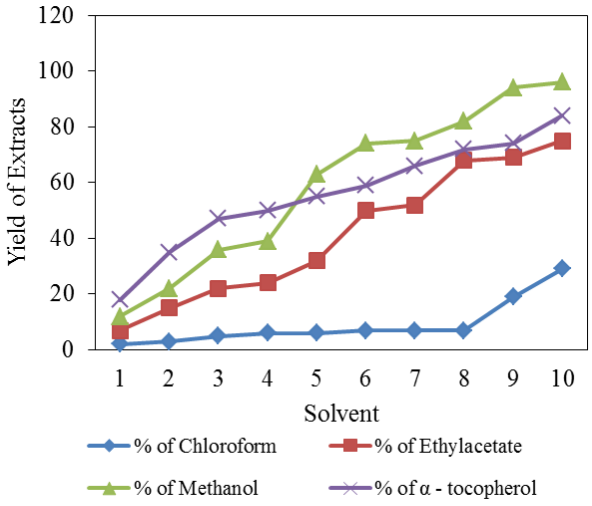

Figure1. DPPH assay

Metal chelating activity

Presence of transition metal ions in a biological system could catalyse the Haber–Weiss and Fenton-type reactions, resulting in generation of hydroxyl radicals (OH). However, these transition metal ions could form chelates with the antioxidants, which result in the suppression of OH generation, and inhibit ion of per oxidation processes of biological molecules. In this assay, the presence of chelating agents in the extract of *A. malabarica* disrupts the ferrozine - Fe2+ complex formation, thus decreasing the red colour. The metal ion scavenging effects of ethyl acetate extract. It is reported that chelating agents are effective as secondary antioxidants because they reduce the redox potential, thereby stabilizing the oxidized form of the metal ion. The data presented in this study indicated that *A. malabarica* ability for iron binding and could reduce the generation of hydroxyl radicals (Figure 2) .

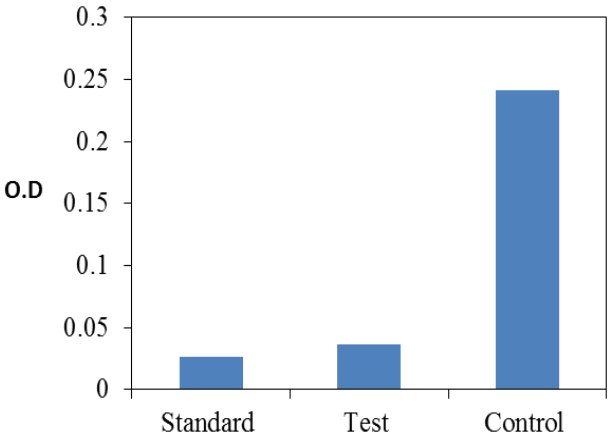

Figure 2. Metal Chelating Activity

Gas chromatography coupled with mass spectrometry

The major components and their retention times are summarized as follows. Among the identified compounds, some of them are known for their interesting biological capacity; 0.39% NEOPHYTADIENE, 1.43% 6, 10, 14-TRIMETHYL-2- PENTADECANONE, 5.25% 3, 7, 11, 15-TETRAMETHYLHEXADEC-2-EN-1-OL, 0.89% cis-13-Docosenamide, 0.44% Tetracosamethyl-cyclododecasiloxane, 5.34% Squalene, 1.84% LONGIPINAN, TRANS-, 1.07% 6-(3-ACETYL-1- CYCLOPROPEN-1-YL)-3-HYDROXY-6-METHYL-2-HEPTANONE, 0.49% SILICONE OIL, 0.59% 3, 7, 11-TRIMETHYL-6,10-DODECADIEN-3-OL, 1.98% (-)-Globulol, 5.63% Methyl erucate, 5.70% Vitamin E acetate, 2.67% Baccharis oxide, 1.91% Methyl cis-10-heptadecenoate, 30.35% 2-Cyclohexen-1-one-4-carboxylic acid, 4-(3,7,11-trimethyl-2,6,10-dodecatrien-1-yl), 4.77% ISOCHIAPIN B, 4.91% Dotriacontyl trifluoroacetate, 5.67% Methyl cis-13,16-Docosadienate, 7.88% Fumaricacid, tetradec-3-enyl tridecyl ester, 10.79% 1, 54-DIBROMOTETRAPENTACONTANE (Figure 3).

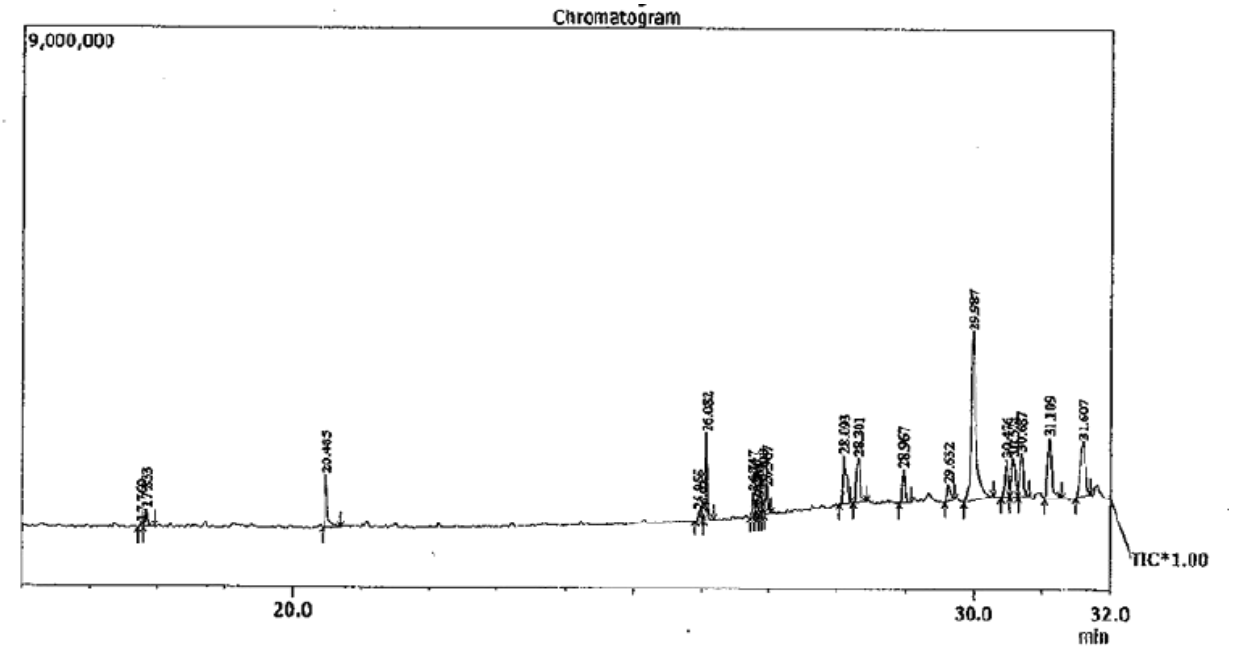

Figure 3. GC-MS Peaks

Wound healing assay

The wound healing assay, was conducted using MTT assay on VERO cell line and the results for VERO cell line IC50 values showed highest activity *A. malabarica* was found to be 50.9 µg/ml (Figure 4) picture's showed the wound healing process on VERO cell lines.

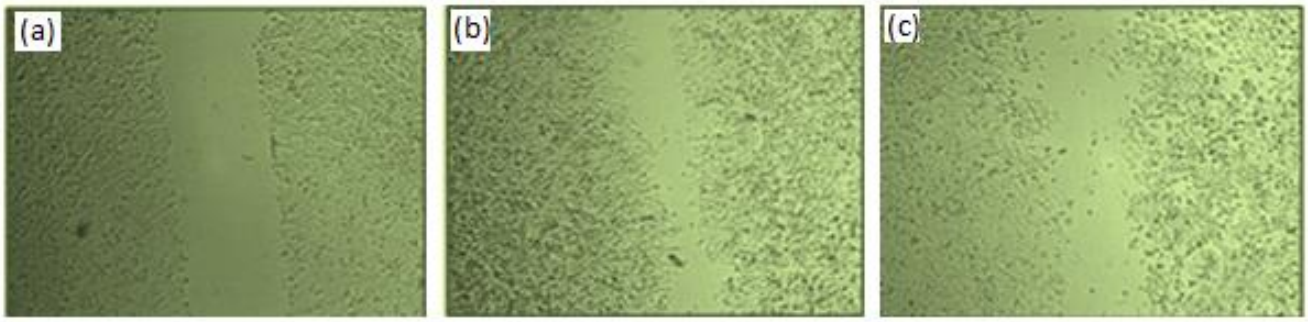

Figure 4. Wound Healing Assay (a) Wound created on VERO Cell line at 0 Hrs (b) Wound Healed at 4 Hrs (c) Wound Healed at 8 Hrs

Discussion

The *A. malabarica* is an important medicinal plant found in Indian traditional medicine. The dried leafs were extracted with solvents and were recovered using soxhelet apparatus and the plant extracts showed an ample variety of antioxidant property which is evident from the GCMS analysis study. The *A. malabarica* methanolic extract was evaluated using scavenging assay and total antioxidant capacity. The Cytotoxity study on VERO cell lines by means of MTT assay showed broad variety of wound healing properties of *A. malabarica*, in the MTT assay test it showed cell viability of 50.09%. Results of the present study come in line with that of [11] which showed the wound healing property of *A. malabarica* plant on wound excised rabbits. This proved the theory that *A. malabarica* has effective wound healing property due to the existence of high tannin content which is accountable for the wound contraction and ephithelization hence it can be inferred that the MTT studies on VERO cell lines showed superior efficient wound healing process.

Conflict of interest

The authors declare that there is no conflict of interests regarding the publication of this paper. Also, they declare that this paper or part of it has not been published elsewhere.

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