IJPSR (2016), Vol. 7, Issue 7 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 16 February, 2016; received in revised form, 19 March, 2016; accepted, 04 May, 2016; published 01 July, 2016

PHYTOCHEMICAL INVESTIGATION FOR THE WOUND HEALING POTENTIAL OF A NOVEL COMPOUND ISOLATED FROM *MENTHA PIPERITA* L. LEAVES

Iram Rais[* 1] and Mohammad Ali [2]

Department of Biochemistry [1], Faculty of Science, Jamia Hamdard, New Delhi - 110062, India.
Department of Pharmacognosy and Phytochemistry[2], Faculty of Pharmacy, Jamia Hamdard, New Delhi – 110062, India

Keywords:

Mentha piperita;compound-1; 13C NMR; collagen; hydroxyproline; wound healing activity

Correspondence to Author:
Iram Rais

Department of Biochemistry,
Faculty of Science,
Jamia Hamdard, Hamdard Nagar,
New Delhi - 110062. India.

Email: iram_khan2222@rediffmail.com

ABSTRACT: *Mentha piperita*is currently one of the most economically important aromatic and medicinal crops in all over the world. Phytochemical investigation of the leaves of *Mentha piperita* (L.) (Lamiaceae) yielded a new compound, which is decarboxyrosemarinic acid galactoside. Formulate as 3', 4'-dihydroxy-β-phenyl ethyl caffeate-4-(3'''-menthyl)-4'-β-D-galacto pyranoside. 13C NMR, 1H NMR, FABMS, IR were used for structural characterization. Wound healing responses of this compound was evaluated from biochemical as well as biophysical parameters and analyze the role of isolated compound. The parameters studied included rate of wound contraction and the period of epithelialization in excision wound model. Tissues were removed on different days of intervals and subsequently analyzed for specific assays Tensile strength in incision wound model was assessed along with histopathological examinations. Compound-1was found to increase the cellular proliferation and collagensynthesis at the wound site, as indicated by increases in amounts of DNA synthesized, protein content. Compound-1treatment was also shown to decrease the levels of lipid peroxides (LPs), while the activity of enzymes superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx), were significantly found to be increased when compared to the control. This study demonstrates and validates the efficacy of *Mentha piperita* isolated compounds on wound healing activity.

INTRODUCTION: The phenomenon of wound healing is a coordinated cascade of cellular responses. That is initiated by trauma and often terminated by scar formation. It proceeds in three well known stages: inflammation, proliferation and remodelling. The proliferative phase is characterized by angiogenesis, collagen deposition, granulation tissue formation, epithelialization and wound contraction. . In angiogenesis, new blood vessels grow from endothelial cells.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.7(7).2781-94

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.7 (7).2781-94

In fibroplasia and granulation tissue formation, fibroblasts grow and form a new, provisional extracellular matrix by excreting collagen and fibronectin. Collagen, the major component which strengthens and supports extracellular tissue, contains substantial amounts of hydroxyproline, which has been used as a biochemical marker for tissue collagen. [1] Such wounds are difficult and frustrating to manage. Current methods used to treat chronic wounds include debridement, irrigation, antibiotics, tissue grafts and proteolytic enzymes, which possess major drawbacks and unwanted side effects [2]. *Mentha piperita* is commonly known as Peppermint. Peppermint is taken internally as a tea, tincture, oil, or extract, and applied externally as a rub or liniment. About eighty five constituents of peppermint have been identified, and a further fourty are unidentified.

Flavonoids luteolin and its 7-glycoside (cynaroside) menthoside, isorhoifolin and others including a number of highly oxygenated flavones have been reported. [3, 4] Triterpene, squaline, α amyrene, ursolic acid and sitosterol and other constituents azulene and minerals are also reported.[5] Sugar moieties found in naturally occurring saponins are quite diversified, usually they are D-glucose, D-galactose, D-xylose, D-fucose, L-rhamnose, L-arabinose and D-glucuronic acid. [6] Many saponins are present in higher plants in the form of glycosides of complex alicyclic compounds. The biological and pharmacological properties exhibited by saponins are quite diversified (haemolytic, cytotoxic, antitumor, anti-inflammatory, molluscicidal) and have been extensively reviewed over the past few years. [7, 8] Several species widely used in traditional system of medicine and China, especially those having a triterpenoid aglycone. [9] The classical and advent of chromatographic techniques are also involved in isolation of triterpenoid saponins. The unique chemical nature of saponins demands tedious and sophisticated techniques for their isolation, structure elucidation and analysis. [10, 11, 12] The present study has been undertaken to ascertain the effect of Compound-1 on experimentally induced wounds in rats.

Objective:

To analyse the wound healing response of an isolated compound "Menthyl teucrol glycoside" and its structural characterization.

MATERIALS AND METHODS:

Melting points were determined on Perfit melting apparatus. Ultra violet spectra were recorded on Lambda bio 20 spectrometer in methanol. Infrared spectra read on a Biorad FTIR spectrophotometer using KBr pellets. 1H-NMR spectra were observed on advance drx 400-Brucker spectrospin 400 MHz, employing Tetramethyl silane as an internal standard. 13C-NMR spectra were recorded on advance drx 400, Brucker spectrospin 100 MHz in 5 mm spinning tubes at 27^0C. Mass spectra were scanned by FAB Mass Jeol instrument equipped with a direct inlet probe system.

Ethical statement: This project was approved by the Institutional Animal Ethical Committee, animals were maintained under standard conditions in an animal house approved by the Committee for the purpose of control and supervision on experiments on animals (CPCSEA) 172/2000.

Experimental Animals:

Male albino Wistar rats weighing 150-200 g were used for the experiments. They were individually housed and maintained on normal food and water ad libitum. Animals were periodically weighed before and after experiments. All the animals were closely observed for any infection and those which indicated signs of infection were separated and excluded from the study. Rats were randomly distributed into three groups of six for both models.They were subjected to starvation for 12 hours prior to the experiment. Group I served as Control, which received normal saline. Group II served as the standard which was treated with framycetin sulphate cream, group III as test, received pure compound mixed with double distilled water at a dose of 30 mg/kg body weight respectively.

Collection and Identification of Plant Material:

The leaves of *Mentha piperita* were procured from Herbal garden Jamia Hamdard, New Delhi, sample was identified by National Institute of Science Communication and Information Resources (NISCAIR), New Delhi, India. A voucher specimen of this sample was deposited in the Raw Materials Herbarium and Museum, NISCAIR with reference number Consult/-2008-09/1169/201.

Extraction, separation and purification of the compounds:

The air dried, powdered, defatted leaves of 1kg *Mentha piperita*, were extracted exhaustively in a Soxhlet apparatus with aqueous ethanol (4:1), the extract was further dried under reduced pressure and dissolved in water saturated with n-butanol (10 liters). Ethyl acetate was added to the n-butanol solution to precipitate glycosides.

The mixture was filtered using a fine muslin cloth followed by filter paper (Whatman No 1). The filtrate was placed in an oven to dry at 40^0C. The clear residue obtained was used for the study. The residue was subjected to preliminary phytochemical analysis.

Confirmatory test of saponins:

Extract (300 mg) was boiled with 5 ml water for 2 min; the mixture was cooled and mixed vigorously and left to stand for 3 minutes. Frothing indicates the presence of saponins. [13]

The dry residue was dissolved in minimum amount of ethanol and silica gel (20-120 mesh, 100g) added to form slurry. The slurry was dried and subsequently loaded on silica gel column prepared in chloroform. The column was run with chloroform and chloroform-methanol (99:1, 49:1, 19:5, 9:1, 4:3, 3:1 and 1:1 v/v) to isolate the following compound: Menthyl teucrol Glycoside. Elution of the column with chloroform: methanol (9:1) furnished light brown crystals of Compound-1, recrystallized from chloroform-methanol (1:1). Yield: 6 g (6.75 % yield), R_f : 0.54 (chloroform-methanol , 9:1), Mp: (149-150^{0}C), UV λ_{max} (MeOH): 250, 290,332 nm (log ξ 1.2, 6.3, 5.8), I Rν_{max} (KBr): 3425, 3366, 3255, 2933, 2845, 1720, 1605, 1517, 1446, 1386, 1284, 1163, 1074 cm^{-1}.^{1}H NMR (MeOD): **Table 1**

^{13}C NMR (MeOD): Table-1, +ve FAB MS: m/z (ret. Int.): 617 $[M+H]^+$ ($C_{33}H_{45}O_{11}$)(2.5)

TABLE 1: ^{1}H NMR AND ^{13}C NMR SPECTRAL DATA OF MENTHYL TEUCROL GLYCOSIDE (COMPOUND-1)

Position	^{1}H NMR	^{13}C NMR
1	--	126.31
2	7.05 d (1.4)	115.01
3	---	144.69
4	----------	148.26
5	6.81d (8.1)	116.29
6	6.93 dd (8.1, 1.4)	121.86
7	6.28 d (15.6)	146.24
8	7.55d (15.6)	113.94
9	----	167.30
1’	----	126.24
2’	6.63 d (1.5)	113.24
3’	-----	143.76
4’	------	145.32
5’	6.71 d (8.0)	113.22
6’	6.77 dd (8.0, 1.5)	120.53
7’	2.28 d (12.6), 2.21 d(11.6)	36.67
8’	3.54 d (12.6), 3.5 d (11.6)	62.02
1’’	5.32 d (7.1)	102.01
2’’	3.62 dd (7.1, 6.3)	33.03
3’’	33.70 m	72.46
4’’	3.76 m	70.21
5’’	4.34 m	76.69
6’’	3.02 d (7.2), 3.01 d(2.4)	61.37
1’’’	2.02 m	29.36
2’’’	2.78 m, 2.65 m	22.44
3’’’	3.86 dd (5.3, 8.9)	76.42
4’’’	2.14 m	34.37
5’’’	1.77 m, 1.70 m	20.18
6’’’	1.60 m, 1.49 m	18.65
7’’’	1.01 d (6.5)	15.02
8’’’	1.94 m	26.56
9’’’	1.20 d (6.1)	12.93
10’’’	1.18 d (6.0)	13.99

Coupling constants in Hertz are given in parentheses

Biophysical Parameters:

Excision wound induction:

Animals were anesthetized with ketamine hydrochloride prior and during creation of wounds. The excision wound was created as per the method of. [14] The dorsal fur of the animals was shaved off the anticipated area of the wound to be created was outlined on the back of the animals with a full thickness of the excision wound of one square cm area of a 2mm depth was created. Animals were randomly divided in to two group of six each. To the control group animals normal saline was applied. The test group rats were given pure compound 30 mg/kg body weight daily until complete healing. Since an average the wound closure rate was assessed by vernier caliper on day one to complete healing.

The day of scar falling off, after wounding, without any residual raw wound was considered as the time until complete. Epithelialization was performed as standardized by. [15] Contractions were studied by vernier calipers. Wounds were measured on fourth, eighth and twelfth day respectively until they were completely covered with epithelium. Wound contraction (WC) was calculated as a percent change in the initial wound size as follows:

WC (%) = Initial wound size – specific day wound size / Initial wound size

Epithelialization period was monitored by evaluating the number of days required for scar to fall away, leaving no raw wound area behind.[16]

Incision wound induction:

Incision wound was created as the methodology adopted from.[17] The animals were anesthetized by intraperitonial injections of 8mg/ml ketamine hydrochloride and hair clipped from the entire trunk. They were shaved before preceding the wounding protocol. A 75% alcohol was employed for skin preparation by a toothed forceps to elevate the starting point of the incision at the lower left posterior flank, one cm lateral to the spine. A sharp-blunt scissor was employed to make a small cut through the whole thickness of the skin. The blunt tip of the scissor was inserted through the cut in the loose aereolar tissue. The forceps was repositioned to hold the tissue at the incision site and "pushcut" the skin with scissors along the peraspinous longitudinal line until a four cm incision was made. Animals were positioned with their head to left of the operator. Further, both ends of the wound were held with forceps to create a wound pocket and test material applied. A grasp with three evenly spaced cotton bandages was made. Bandage was removed after five days of wounding. Pure compound was applied twice daily.

Histopathology:

For histopathology, a portion of the healed skin or a portion during the healing stage was excised and processed for microscopy. This allowed fixing of the specimens in 10% neutral buffered formalin solution, embedding them in paraffin slicing sections 5-6 μm in thickness, and staining with haematoxylin and eosin. [18] The sections were qualitatively assessed under the light microscope and graded with respect to edema, infiltration, monocytes, necrosis, fibroblast, proliferation, collagen formation and epithelialization.

Tensile strength:

Tensile strength was measured as the method adopted by [19], which has been explained as the resistance of the skin to break under tension and may indicate in part the quality of the repaired tissue. For this purpose the newly repaired tissue including scar was fixed under the appropriate tension for measurement. The wound tensile strength was determined in all groups on the twentieth day by texture analyzer (TA XT2). [20]

Rats were lightly anesthetized with ether. The skin was removed with one cm on each side of the wound. Measurements were performed employing the following equation.

Tensile strength = Breaking load (force)/Area

Area= Thickness× Width

Biochemical Parameters:

Protein and DNA of wet granulation tissue (100 mg wet wt of tissue) were extracted in a 5% trichloroacetic acid (TCA) solution. The protein was assayed according to the method described by. [21, 22]0.1ml of sample tissue, 0.1ml chilled TCA (10%) was added. Samples were allowed to

incubate for 30 minutes for protein precipitation and centrifuged at 3000 rpm for 10 minutes. The supernatants were decanted and discarded. The pellet was dissolved in 2 ml of 1N NaOH and kept at 30 ^{0}C in a water bath for 20 minutes. Aliquots of 0.2 ml in duplicate were taken and 0.8ml water and 2.5 ml of alkaline copper sulphate reagent was added. Following 10 minutes incubation at 25^{0}Ctemperature after addition of alkaline copper sulphate reagent to allow complex formation, 0.25 ml of folins reagent was added, and incubated for30 minutes at room temperature, optical density of the final product measured at 660nm.

Hexosamine estimation:

Hexosamine was determined as per protocol adopted from. [23] Aminosugars or hexosamine formed when an amino sugar is introduced in hexose. Thus glucose with NH_2 and C-2 forms glucosamine. This compound is extensively found in complex polysaccharides in the form of acetyl derivative N-acetyl glucosamine. This is the constituent of some glycoproteins in connective tissues. Therefore we considered glucosamine as a standard for the measurement of hexosamine. This is a colorimetric method and this method depends upon the colour which develops when pyrroles are condensed with p-dimethylaminobenzaldehyde.

The conversion of glucosamine into pyrrole derivatives Ethyl ester of 2-methyl-5-tetrahydroxybutylpyrrole-3-carboxylic acid and 3-Acetyl-2-methyl-5-tetrahydroxybutylpyrrole by the action of ethyl acetoacetate and acetyl acetone respectively, when glucosamine hydrochloride is boiled in alkaline solution with either ethyl acetoacetate or acetyl acetone, the resulting solutions on treatment with Ehrlich reagent in the presence of alcohol, develop a stable red colour. The colour obtained with the condensation product of acetyl acetone was found to be more intense and for this reason was selected as a basis for the colorimetric determination of Hexosamine. This was carried out in test tubes at a graduation mark volume of 10 ml. The standard solution contained between 0.5-3.0 mg of glucosamine hydrochloride concentration that was pipetted in the test tubes and acetyl acetone reagent added in a test tube by the side, washed with 1 ml double distilled water. After that tubes were heated in boiling water. Cooled and alcohol added to within about 2 ml of the 10 ml graduation mark.

Samples of varying concentrations of the standard were taken for analysis. The solutions were treated with 1ml of freshly prepared 2% acetylacetone in 0.5M Na_2C0_3 in capped tubes and kept in a boiling water bath for 15min. After cooling in tap water, 5ml of 95% ethanol and 1ml Ehrlich's reagent were added and mixed thoroughly. The purple red color developed was read after 30min at 530nm.

Total collagen estimation:

The total collagen estimation was determined by. [24] Collagen was estimated with respect to L-Hydroxyproline. Samples of varying concentrations were taken for analysis. Hydroxy proline was oxidized by adding 1ml of Chloramine T to each tube. The contents were mixed thoroughly by mixing and allowed to stand for 20minutes at room temperature. Adding 1ml of 70% perchloric acid to each tube then destroyed the layer of Chloramine T. The contents were allowed to mixed and stand for 5 minutes. Finally 1ml of PDAB (Para Dimethyl Amino Benzaldehyde) solution was added andcolour developed read spectrophotometrically at 557 nm. The collagen content was then calculated by multiplying the hydroxyproline content by the factor 7.46 [24] and was expressed as mg/100 mg of dry weight of the sample.

Catalase:

Catalase activity has been measured as per protocol adopted from. [25] The reaction mixture consisted of 1.95 ml of phosphate buffer (0.1 M, PH 7.4), 1 ml H_2O_2 (0.09 M) and 0.05 ml PMS (10% w/v) in a final volume of 3 ml. Change in absorbance after 3 minutes was recorded at 240nm. Catalase activity was calculated in terms of nmol H_2O_2 Consumed /min/mg protein.

Superoxide dismutase:

SOD activity was measured as per protocol adopted from. [26] Pyrogallol auto-oxidation by superoxide radical (O_2) generated by univalent reduction of oxygen is inhibited by superoxide dismutase (SOD).

SOD

$2O_2 + 2H \longrightarrow H_2O_2 + O_2$

For preparation of tris buffer 50 mM Tris and 1 mM EDTA dissolved in double distilled water, pH adjusted to 8.5 by HCl and for pyrogallol solution, 20 mM pyrogallol dissolved in double distilled water. The solution was prepared at the time of assay. In control test tube 2.9 ml tris buffer and 0.1 ml pyrogallol was added and in test sample 2.8 ml tris buffer, 0.1 ml pyrogallol and 0.1 ml PMS sample was taken. After induction period of 90 seconds, absorbance was recorded first in control and then in test every 30 seconds for 3 minutes at 420 nm. Pyrogallol auto-oxidation per 3 ml assay mixture and was given by the formula.

Unit of SOD per ml of sample = A-B/Ax50 X 100x10 (dilution factor)

Where, A = Difference of absorbance in 1 minute in control, and B = Difference of absorbance in 1 minute in test sample. Results expressed in units/mg protein. Protein was estimated by Lowry's method.

Glutathione peroxidise (GPx):

Specific activity of the enzyme was measured according to the procedure described by. [27] The reaction mixture in a 3.0 ml cuvette consisted of 1.5 ml of phosphate buffer (0.05M, pH 7.0), 0.1mM EDTA (3.722mg in 10 ml of distilled water), 0.1 ml of 1mM NaN_3 (1.3 mg in 20 ml of distilled water), 0.1 ml of 0.2mM NADPH (freshly prepared by dissolving 1.67 mg in 10 ml of 0.05M phosphate buffer of pH 7.0), 0.01 ml of 0.25 mM H_2O_2 and 80 µl of PMS (10% w/v) in a final volume of 2.0 ml. the activity was measured in terms of decrease in absorbance at 340 nm suggestive of disappearance of NADPH at an interval of 30 sec for 3.0 minute at room temperature. The enzyme activity was calculated as n mol of NADPH oxidized /minute/mg protein by employing molar extinction coefficient of $6.22\times10^{3}M^{-1}cm^{-1}$.

Lipid peroxide (LPO):

Lipid peroxide was estimated as per protocol adopted from. [28] Lipid peroxide formation was detected by thiobarbituric acid reaction and expressed as malondialdehyde (MDA) equivalent. Acetic acid 1.5 ml (20%) Ph 3.5, and 1.5 ml TBA (0.8%) and 0.2 ml sodiumdodesyl sulphate (8.1%) were added to 0.1 ml of processed tissue sample. The mixture was then heated at 100^{0}C, cooled with tap water and 5 ml of n-butanol: pyridine (15:1% v/v) 1 ml of distilled water was added. The mixture was allowed to mix vigorously. After centrifugation at 4000 rpm for 10 minutes the organic layer was withdrawn and absorbance was measured 532 nm using a spectrophotometer (Shimadzu-160, Japan). The amount of MDA formed in each sample was expressed as the n mol MDA formed $h^{-1}mg^{-1}$ protein by using a molar extinction coefficient of $1.56\times105\ M^{-1}cm^{-1}$.

DNA isolation and quantification by kit (Hipure™ Mammalian Genomic DNA Purification Kit):

DNA isolation was performed by. [29, 30] Make a series of dilution from DNA stock solution in buffered saline. Add 4 ml DPA to each standard and Tests sample. Prepare a blank. Place all the tubes in boiling water bath for 10 minutets till the blue colour developed in the tubes. Cool all the test tubes by placing them in to the water tray. Take O.D. at 600 nm.

The whole procedure of collagen fractionation (Neutral-Salt-Soluble Collagen, Acid-Soluble Collagen and Insoluble Collagen) was done by the method described. [31]

Preparation of Collagen:

The healed skin was cleaned off, fat and muscle, cut into small pieces and homogenised in an Ultra-Turrax homogeniser.

Neutral-Salt-Soluble Collagen:

All procedures carried out at 4^{0}C. The frozen samples were thawed and cut out in small pieces using a scalpel. These pieces were homogenized for 3 days with 10 volumes of NaC1-phosphate buffer (pH 7.4) containing 28.1 g NaC1, 1.96 g Na_2HPO_4 12 H_2O and 0.16 g KH_2PO_4 per litre. The homogenate was then squeezed using cheesecloth to remove any non required particle. Residue was washed with distilled water for one day. The suspension was centrifuged for 2 hours at 22,000 rev./min (Rotor 30 of Spinco Centrifuge Model L). This was repeated four times. The supernatants were pooled and salted out to isolate and purify the collagen. Neutral-salt-soluble collagen was

precipitated from the supernatant by adding NaCl to a final concentration of 20-25O/, (w/v). The collagen was collected by centrifugation (22,000 rev./min, 2 h) and redissolved in citrate buffer, pH 3.7 (10.9 g citric acid monohydrate, 100ml 0.1 M NaOH and 50 ml 0.1 M HCl per litre). After a 48-hours dialysis against the same buffer the samples were centrifuged again and the collagen reprecipitated from the supernatant. The precipitate was redissolved in 0.050/, (v/v) acetic acid and lyophilized. The samples were stored at 5^0C over calcium chloride.

Assessment of wound healing activity:

Evaluation of wound healing activity was performed employing two types of models: the excision and incision wound induced model.

Statistical analysis:

The means of wound area measurement at different time intervals, period of epithelialization, wet and dry weight and collagen content between the test and control groups were compared using nonparametric Mann–Whitney U-tests. Data were analyzed using SPSS (Version 12.0, Chicago, USA) and P-value was set as <0.05 for all analyses.

RESULTS AND DISCUSSION:

Compound-1 named, Menthyl teucrol glycoside was obtained as a light brown crystalline mass from chloroform-methanol (9:1) eluants. It gave positive tests for phenols and exhibited a fluorescent blue spot on TLC under UV lamp changing to bright yellow colour on fuming with ammonia and UV absorption maxima at 250, 290, 332 nm typical for caffeote ester. [32] IR spectrum showed characteristic absorption bands for hydroxyl groups (3425, 3366, 3255 cm^{-1}), ester group (1720 cm^{-1}) and aromatic ring(1605, 1517, 1074 cm^{-1}). On the basis of FAB mass and ^{13}C NMR spectra, the molecular weight of Compound-1 was established at m/z 616 consistent to the molecular formula of glucosidic menthyl teucrol, $C_{33}H_{45}O_{11}$.

The ^{1}HNMR spectrum of Compound-1 exhibited four one-proton doublets at δ 6.81 (J=8.1 Hz) 6.71 (J=8.0 Hz), 7.05 (J=1.4Hz) and 6.63 (J=1.5Hz) assigned correspondingly to ortho-coupled H-5, H-5’ and meta -coupled H-2, H-2’ protons, respectively. Two one-proton double doublets at δ 6.93 (J=8.1, 1.4 Hz) and 6.77 (J=8.0, 1.5 Hz) w ere ascribed to ortho- meta- coupled H-6 and H-6’, respectively suggesting ABX system of both the rings. Two one- proton doublets at δ 6.28 and 7.55 with coupling interactions of 15.6 Hz were attributed to trans oriented venylic H-7 and H-8 protons, respectively. Two one- proton doublets at δ 3.54 (J=12.6 Hz) and 3.59 (J=11.6 Hz) were due to oxygenated methylene H_2-8’ protons. A one-proton doublet at δ 5.32 (J=7.1 Hz) was accounted to anomeric H-1’’ proton.

The other sugar protons appeared between δ 4.34 to 3.01. A one- proton double doublet at δ 3.86 (J=5.3, 8.9 Hz) was accommodated to α-oriented oxygenated methine H-3’’’ proton. Three doublets at δ 1.01 (J=6.5 Hz), 1.20 (6.1 Hz) and 1.18 (J=6.0 Hz), integrated for three protons each, were associated with secondary C-7’’’, C-9’’’ and C-10’’’. methyl protons, respectively. The remaining methine and methylene protons resonated from δ 2.28 to 1.49. These data supported incorporation of glycosidic cafeate ester of 3, 4 dihydroxy- β-phenyl ethanol linked with menthol.

The ^{13}C NMR spectrum of Compound-1 exhibited signals for ester carbon at δ 167.30 (C-9) aromatic and venylic carbons between δ 148.26 - 113.22 anomeric carbon at δ 102.01 (C-1’’), other sugar carbons from δ 76.69 to 61.37, oxygenated methine carbon at δ 76.42 (C-3’’’), oxygenated methylene carbon at δ 62.02 (C-8’) and methyl carbons at δ 15.02 (C-7’’’), 12.93 (C-9’’’) and 13.99 (C-10’’’). The ^{13}C NMR spectral data were compared with rosemeric acid [33]and teucrol. [34] The carbon signals of the sugar unit were comparable to galactoside chain. [34, 35]

The normal positions of the sugar carbons in the ^{13}C NMR spectrum suggested that menthol was linked to the phenolic carbon and not to the sugar unit. Acid hydrolysis of Compound-1 yielded cafferic acid, 3, 4-dihydroxy-β-phenyl ethanol, galactose and menthol, co-TLC comparable. On the basis of the foregoing discussion the structure of Compound-1 was formulated 3’, 4’-dihydroxy-β-phenyl ethyl caffeate-4-(3’’’-menthyl)-4’-β-D-galacto pyranoside. This is a new decarboxyrosemarininc acid galactoside.

2.1 Structure of Compound-1 Menthyl teucrol glycoside:

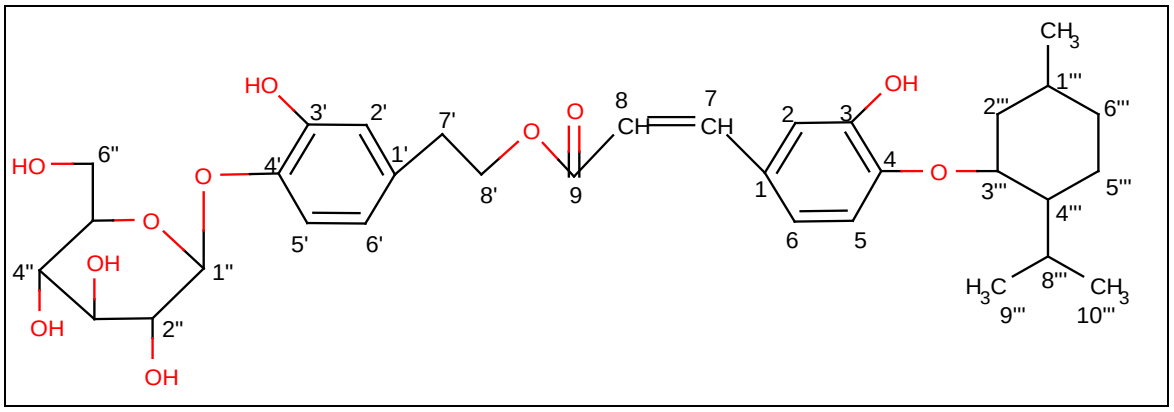

TABLE 2: WOUND CONTRACTION IN MM^2 CONTROL AND COMPOUND-1 TREATED GROUP AT DIFFERENT DAYS

Treatments	Post wounding reduction											
	0 day	Fourth day		Eighth day		Twelfth day		Fourteenth day		Sixteenth day		Eighteenth day	
	Mean	Mean	± SE	Mean	± SE	Mean	± SE	Mean	± SE	Mean	± SE	Mean	± SE
Control	1.0	0.992	0.003	0.870	0.019	0.460	0.022	0.273	0.022	0.100	0.022	0.025	0.015
Standard	1.0	0.947	0.021	0.737	0.033	0.312	0.015	0.058	0.022	0.000	0.000	0.000	0.000
Compound-1	1.0	0.850	0.018	0.662	0.005	0.278	0.032	0.000	0.000	0.000	0.000	0.000	0.000

TABLE 3: PERIOD OF EPITHELIALIZATION FOR CONTROL AND COMPOUND-1 TREATED GROUP AT DIFFERENT DAYS

Treatments	Days	±SE
Control	17.83	0.31
Standard	14.00	0.26
Compound-1	13.67	0.42

TABLE 4: TENSILE STRENGTH OF CONTROL AND COMPOUND-1 TREATED ANIMALS ON TWENTIETH DAY TISSUE

Treatments	Mean(grams)	SE ±
Control	2585.02^f	±311.00
Standard	8654.24^b	±522.42
Compound-1	6167.09^e	±506.11

TABLE 5: TOTAL COLLAGEN, HEXOSAMINE, PROTEIN, DNA IN CONTROL AND COMPOUND-1 TREATED ANIMALS ON THE EIGHTH DAY OF HEALED TISSUE

	Fourth day		Eighth day		Twelfth day	
	Mean	SE ±	Mean	SE ±	Mean	SE ±
Total Collagen (100 mg/mg dry tissue)						
Control	1.78	±0.23	5.49	±0.14	2.74	±0.20
Standard	4.17	±0.22	8.58	±0.30	6.40	±0.22
Compound-1	7.43a	±0.17	10.72a	±0.26	6.90a	±0.29
Hexosamine (µg/100 mg dry tissue)						
Control	431.50	42.37	527.67	20.77	308.33	3.64
Standard	1926.67	47.45	1192.67	42.35	748.33	21.04
Compound-1	2065.67a	43.72	1271.62a	23.89	790.62b	15.61
Protein (mg/100 mg wet tissue)						
Control	3.08	0.12	7.362	0.279	5.95	0.29
Standard	7.86	0.55	13.300	0.389	9.055	0.21
Compound-1	9.21a	0.22	14.12a	0.20	10.25a	0.12
DNA (mg/100 mg wet tissue)						
Control	1.96	0.101	5.47	0.32	4.26	0.29
Standard	2.680	0.05	7.06	0.32	6.14	0.17
Compound-1	3.21a	0.12	7.22a	0.22	7.54a	0.21

TABLE 6: LIPID PEROXIDE LEVEL OF CONTROL AND COMPOUND-1 TREATED GROUP ON FOURTH, EIGHTH AND TWELFTH DAY

Treatments	Lipid peroxide in n mol of Malondialdehyde					
	Fourth day		Eighth day		Twelfth day	
	Mean	SE ±	Mean	SE ±	Mean	SE ±
Control	2612.2[a]	37.07	3362.83[a]	32.82	1530.50[b]	34.81
Standard	433.17[d]	15.43	541.67[d]	20	306.33[d]	26.78
Compound-1	475.00[d]	23.69	424.50[e]	12.13	299.50[e]	12.39

TABLE 7: GLUTATHIONE PEROXIDASE IN CONTROL AND COMPOUND-1 TREATED ANIMALS ONEIGHTH DAY HEALED TISSUE

Treatments	Mean	SE ±
Control	64.83[a]	1.02
Standard	80.43[e]	1.54
Compound-1	106.75[d]	1.77

TABLE 8: CATALASE MOLES OF HYDROGEN PEROXIDE DECOMPOSE PER MIN PER MG PROTEIN IN CONTROL AND COMPOUND-1 TREATED ANIMALS ON EIGHTH DAY HEALED TISSUE

Treatments	Mean	SE ±
Control	1.031	0.06
Standard	2.41[b]	0.21
Compound-1	2.83[b]	0.52

TABLE 9: CHANGE IN SUPER OXIDE DISMUTASE SOD UNIT/MG/PROTIEN IN CONTROL AND COMPOUND-1 TREATED ANIMALS ON EIGHTH DAY HEALED TISSUE

Treatments	Mean	SE ±
Control	3.118[f]	0.088
Standard	5.47[e]	0.18
Compound-1	10.685[b]	0.300

TABLE 10: SOLUBILITY PATTERN OF NEUTRAL SALT, ACID SOLUBLE AND INSOLUBLE COLLAGEN IN CONTROL AND COMPOUND-1 TREATED ANIMALS ONEIGHTH DAY HEALED TISSUE

Treatments	Neutral salt (µg/g)		Acid soluble (µg/g)		Insoluble (µg/g)	
	Mean	SE ±	Mean	SE ±	Mean	SE ±
Control	363	17.00	738.67[e]	22.22	2424.67[e]	471.04
Standard	640.62[c]	30.02	1964[c]	41.12	5154.17[d]	208.83
Compound-1	708.33[b]	21.3	1967.5[c]	34.96	5156.83[b]	182.7

TABLE 11: SUSCEPTIBILITY OF INSOLUBLE COLLAGEN IN CONTROL AND COMPOUND-1 TREATED ANIMALS ONEIGHTH DAY HEALED TISSUE IN THE PRESENCE OF DENATURING AGENT 6 M UREA.

Treatments	Mean	SE ±
Control	10.05	0.37
Standard	7.35	0.43
Compound-1	6.09	0.10

TABLE12: ALDEHYDE CONTENT OF ACID SOLUBLE COLLAGEN (µ mol/ 100 mg COLLAGEN) IN CONTROL AND COMPOUND-1 TREATED ANIMALS IN EIGHTH DAY HEALED TISSUE

Treatments	Mean	SE ±
Control	6.19	0.24
Standard	11.04	0.47
Compound-1	8.97	0.16

Wound healing activity of Compound-1:

A significant increase in the wound-healing activity was observed in the animals treated with the Compound-1 compared with those who received the control treatments. **Fig.1** and **2** indicate the effect of the *Mentha piperita* isolated Compound-1 on wound-healing activity in rats inflicted with excision and incision wounds. In both models, the Compound-1 treated animals showed a more rapid decrease in wound size and a decreased time to epithelialization **(Fig.1)** and the incision wound study was also carried out to measure the tensile

strength of the regenerated tissue. Compound-1 treated group indicated significant breaking strength which is more or less similar to the Framycetin treated group.

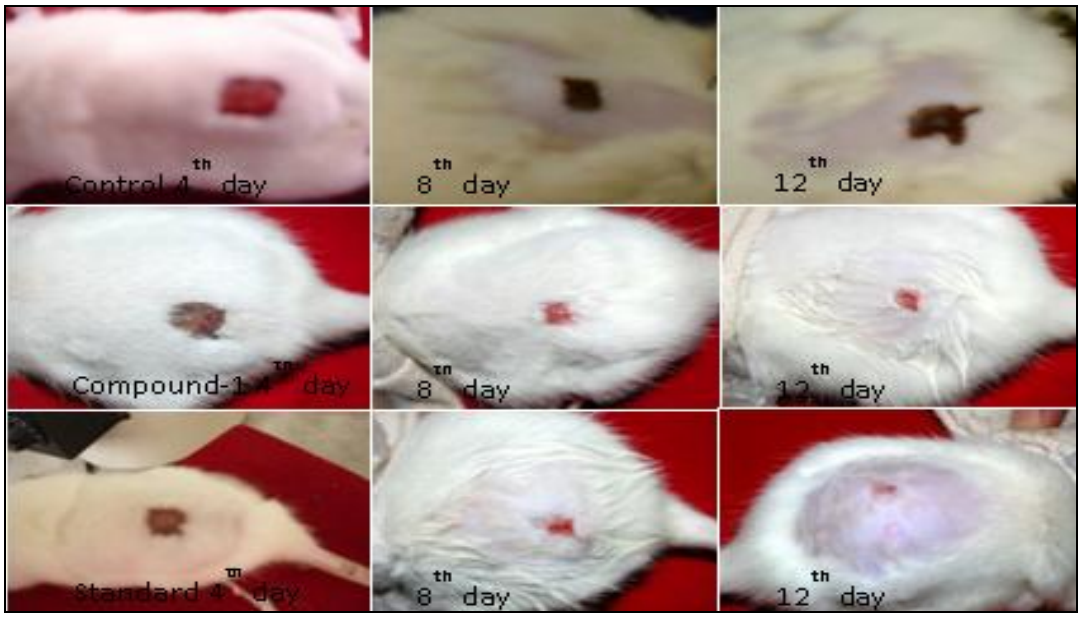

FIG.1: CONTRACTION RATE OF WOUND ON DIFFERENT DAYS OF COMPOUND-1 TREATMENTS AND CONTROL

Collagen:
We observed a significant increase in the collagen content in Compound-1 treated group as compared with control **(Table 5).**

DNA, Protein, and hexosamine:
Compound-1treated group DNA content, Protein and Hexosamine which is the ground substratum for collagen synthesis, increased untill the eighth day **(Table 5).**

Collagen solubility and susceptibility of insoluble collagen:
The solubility pattern of collagen contentdecreased in neutral salt soluble collagen and increased in acid soluble collagen on eighth day **(Table 10)** and aldehyde content of acid soluble collagen was found to be increased as compared to the control in Compound-1 treated group **(Table 12).** The susceptibility of insoluble collagen was observed decreased in Compound-1 treated animal group as compared to control group **(Table 11).**

Tensile strength:
Tensile strength of Compound-1treated group was observed to be increased as compared with control **(Table 4).**

Antioxidant effects:
The antioxidant enzyme levels for Compound-1 treated group, wherein a significant increase in the enzyme parameters is observed in the order of Glutathione peroxidase, SOD, followed by Catalase, as compared with the control group **(Table 7, 9 &8).** The lipid peroxidation as malondialdehyde after treatment of Compound-1, on eighth day granulation tissue was found significantly decreased as compared with control **(Table 6).**

Histopathological Analysis:
Compound-1 on fourth day starting of epithelial layer formation with minimum cleavage at wound site on eighth day Compound-1 showing well-formed epithelial layer with fibroblast proliferation and dense deposition of collagen as compared with control. On twelfth day Compound-1 showing a complete well organized regular epithelial layer and increased blood vessel formation and enhanced proliferation. so we can say Histological sections of granulation tissue from compound treated group showed increased and well-organized bands of collagen, more fibroblasts and few inflammatory cells as compared with Granulation tissue sections obtained from control group revealed more inflammatory cells and less collagen fibers and fibroblasts **(Fig. 2).**

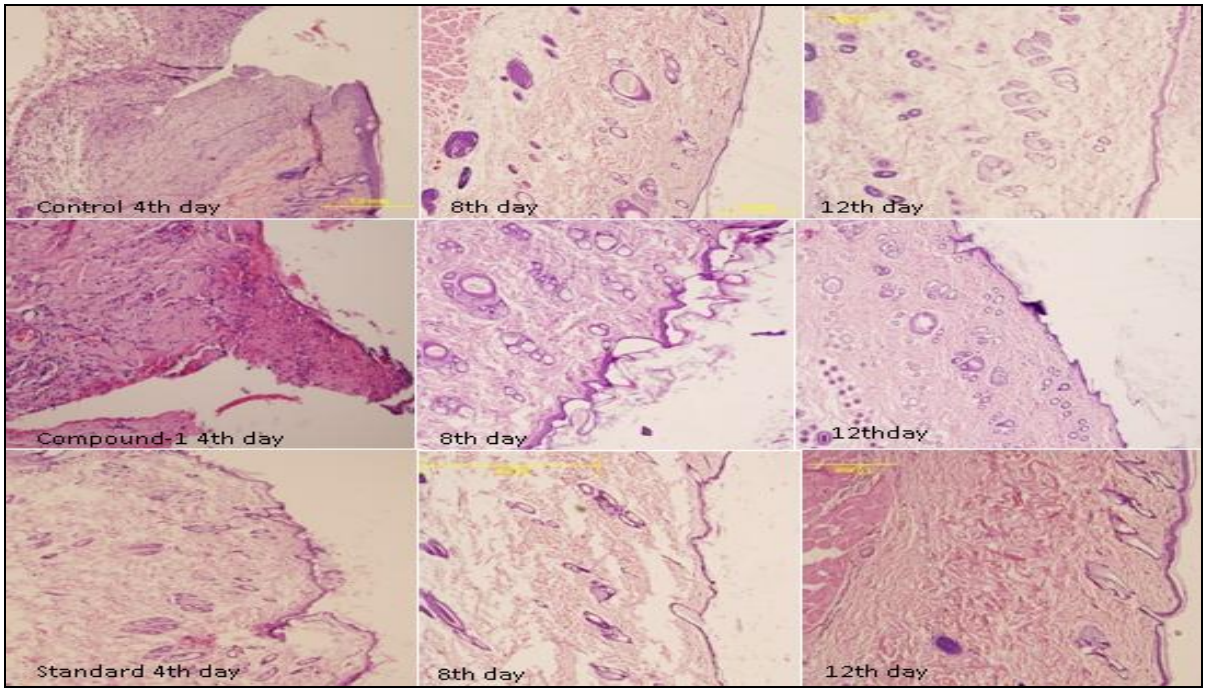

FIG.2: REPRESENTATIVE HISTOPATHOLOGY OF COMPOUND-1 TREATED GROUP WITH CONTROL

Indian medicines based on herbal origin have been the basis of the treatment and cure for various diseases. More than 80% of the world population still depends upon traditional medicines for various skin diseases. Herbal medicine in wound management involves disinfection, debridement, providing a moist environment to encourage the establishment of the suitable environment for natural healing process. [36]

In our study, the Compound-1 treatment on both excision and incision wound models significantly increased the rate of wound contraction, and epithelialization. Granulation tissue formed in the final part of the proliferative phase is primarily composed of fibroblasts, collagen, edema and new small blood vessels. The increase in dry granulation tissue content of wounds in Compound-1 treated animals suggests higher collagen content as compared with control. The compound isolated from *Mentha piperita* may be responsible for promoting the collagen formation at the proliferative stage of wound healing.

Collagen is the major component which strengthens and supports the extracellular tissue, it contains substantial amounts of hydroxyproline, the amount has been used as a biochemical marker for tissue collagen. [1] During granulation tissue formation, as contraction proceeds and resistance increases, fibroblasts differentiate into myofibroblasts. The presence of myofibroblasts is considered to be characteristic of tissue undergoing contraction. [37] The apparent greater number of myofibroblasts in Compound-1 treated wounds may be partially responsible for the fast wound contraction. The protein and DNA content of granulation tissue has indicated the levels of protein synthesis and cellular proliferation, higher proteins and DNA contents (compared to the untreated control) of the treated wound suggested that Compound-1 treated group through an as yet unknown mechanism, stimulated celluler proliferation.

The total collagen / DNA ratioof the granulation tissue from Compound-1 treated group may increase the synthesis of collagen per cell as compared with control. This also indicates hyperplasia in the cell. [38] The protein and DNA content of granulation tissues indicate the levels of protein synthesis and cellular proliferation. It has also been reported that inhibition of proinflammatory markers and stimulation of IL-8 and various growth factors may lead to increased rate of wound contraction which is also involved in wound healing process. [39] Glycosaminoglycans and proteoglycans are synthesized by fibroblasts in the wound area. These substances form a highly hydrated gel-like ground substance, a provisional matrix on which collagen fibers are embedded.

Treatment with hydroalcoholic extract of leaves of *Stachytarpheta jamaicensis* increased the content of ground substance in the granulation tissues. As collagen accumulated, hexosamine levels were increased. It may be seen that the increase in hexosamine content was associated with a concomitant increase in collagen content. [40] Hexosamine is the matrix molecule, which acts as the ground substratum which is responsible for synthesis of new extra cellular matrix.

The early increase in hexosamine showed that the fibroblasts actively synthesize on the ground substratum on which collagen is laid down. It is reported that there is an increase in the levels of these components during the early stages of wound healing, following which normal levels are restored. [41, 42] A similar trend has been observed in Compound-1 treated wound. The level of hexosamine increased up to eighth day. The collagen molecules synthesized at the wound site and become cross linked to form fibers. Wound strength was acquired from both, remodeling of collagen, and the formation of stable intra- and intermolecular cross links. [43]

The amount of acid soluble fraction is higher than that of neutral salt soluble collagen fraction whereas in the Compound-1 treated group,it appears that there is an increased and earlier maturation of collagen fibers in Compound-1 treated group. A significant increase was observed in Compound-1 treated group for tensile strength. The increase in the aldehyde content of the collagen observed in Compound-1 treated wound confirms that the collagen is highly cross linked when compared with the control. [44]

It is also reported that an increase in aldehyde content responsible for greater potential for cross link formation. On comparing the solubility of insoluble collagen in the presence of denaturing agents, we found that the collagen from the Compound-1 treated wounds are less soluble in 6M urea, so we can say that they are highly cross linked than that of the control. [45] It is well documented that the healing process begins with the clotting of blood and completed with remodelling of the cellular integrity of the skin. However, the wound healing process may be prevented by the reactive oxygen species or microbial infection, since the type of cells to be first recruited to the site of injury is the neutrophil which is produced in response to cutaneous injury [46]which has a role in antimicrobial defence and may cause cellular damage by peroxidation of membrane lipids. [47, 48]

CONCLUSION: Our study clearly showed that the compound Menthyl teucrol glycoside, isolated from *Mentha piperita* had wound healing property. This could be a good source of wound healing constituents.

ACKNOWLEDGEMENTS: The authors are highly thankful to CIF Center, Jamia Hamdard, Department of Pharmacognosy & Phytochemistry, Jamia Hamdard and Department of Pathology, AIIMS, New Delhi for providing the instrumentation and experimental infrastructure facility.

CONFLICT OF INTEREST: The authors declare no conflict of interest.

REFERENCES:

1. Kumar R, Katoch SS and Sharma S:β-Adrenoceptor agonist treatment reverses denervation atrophy with augmentation of collagen proliferation in denervated mice gastrocnemius muscle. Ind J Exp Biol2006;44: 371-376.
2. Falanga V: The chronic wound; impaired wound healing and solutions in the context of wound bed preparation. Blood Cells MolDis 2004;32: 88-94.
3. Orani GP, Anderson JW, Sant's Ambrogeo G and Sant's Ambrogeo FB: Upper airway cooling and 1-menthol reduce ventilation in the guinea pig. J Appl Physiol1991; 70, 2080-2086.
4. Rastogi RP, Mehrotra BN: Compendium of Indian Medicinal Plants (1960-1969), CDRI, Lucknow and Publication & Information Directorate, New Delhi, India, 1990.
5. Lucida GM, Wallace JM: Herbal Medicine: In A Clinicians Guide. Pharmaceutical Products Press, New York, London, 1998: 85-86.
6. Bruneton J: Pharmacognosie, Phytochimie, Plantes Médicinales, Editions Technique & Documentation, Paris. 1995.
7. Lacaille-Dubois MA: Biologically and pharmacologically active saponins from plants: recent advances: In Saponins inFood, Feedstuffs and Medicinal Plants. Kluwer Academic Publishers, Pays-Bas, 2000: 205-218.
8. Bachran C, Bachran S, Sutherland M, Bachran D and Fuchs H: Saponins in tumor therapy. Mini Rev Med Chem2008;8: 575-584.
9. Qin GW: Some progress on chemical studies of triterpenoid saponins from chinese medicinal plants. Curr Org Chem1998;2(6): 613-625.

10. Marston A, Wolfender JL and Hostettmann K: Analysis and isolation of saponins from plant material: In Saponins in Food, Feedstuffs and Medicinal Plants. Oleszek W, Marston A (Eds), Kluwer Academic Publishers, Vol. 45,2000: 1-12.
11. Muir AD, Ballantyne KD and Hall TW: LC-MS and LC-MS/MS analysis of saponins and sapogenins–comparison of ionization techniques and their usefulness in compound identification: In Saponins in Food, Feedstuffs and Medicinal Plants. Oleszek W, Marston A (Eds), Kluwer Academic Publishers,Vol. 45,2000: 35-41
12. Schöpke T: Non-NMR methods for structure elucidation of saponins:In Saponins in Food, Feedstuffs and Medicinal Plants. Oleszek W, Marston A (Eds), Kluwer Academic Publishers, Vol. 45,2000: 95-106.
13. Harborne JB: Phytochemical Methods: In A Guide to Modern Techniques of Plant Analysis. Chapman and Hall, London, First Edition,1973.
14. Nayak BS, Anderson M and Pereira LMP: Evaluation of wound-healing potential of *Catharanthus roseus* leaf extract in rats. Fitoterapia2007; 78: 540-544.
15. Manjunatha BK, Vidya SM, Rashmi KV, Mankani KL, Shilpa HJ and Singh SDJ: Evaluation of wound-healing potency of *Vernonia arborea* Hk. Ind J Pharmacol2005; 37: 223-226.
16. Kamath S, Rao SG, Murthy KD, Bairy KL and Bhat S: Enhanced wound contraction and epithelialization period in steroid treated rats: role of pyramid environment. Ind JExp Biol2006; 44: 902-904.
17. Saha K, Mukherjee PK, Das J, Pal M and Saha BP: Wound healing activity of *Leucas lavandulaefolia* Rees. J Ethnopharmacol1997; 56: 139-144.
18. McManus JFA, Mowry RW:Staining Methods, Histologic and Histochemical. Harper 7 Raw, New York, Evanston, London, 1965.
19. Rashed AN, Afifi FU and Disi AM: Simple evaluation of the wound healing activity of a crude extract of *Portulaca oleracea* L. (growing in Jordan) in *Mus musculus* JVI-1. JEthnopharmacol2003; 88: 131-136.
20. Baie SH, Sheikh KA: The wound healing properties of Channa Striatus-cetrimide cream- tensile strength measurement. J Ethnopharmacol2000; 71: 93-100.
21. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ: Protein measurement with the folin phenol reagent. J Biol Chem1951; 193: 265-275.
22. Burton K: A study of the conditions and mechanism of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. Biochem J 1956; 62: 315-323.
23. Elson LA, Morgan WTJ: A colorimetric method for the determination of glucosamine and chrondrosamine. Biochem J1933; 27: 1824-1828.
24. Woessner JFJr.: The determination of Hydroxyproline in tissue and protein samples containing small proportion of this imino acid. Arch Biochem and Biophy1961; 93:440-447.
25. Claiborne A: Catalase activity: In CRC Handbook of Methods for Oxygen Radical Research. Greenwald RA (Ed), CRC Press, Boca Raton, 1985.
26. Marklund S, Marklund G: Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur J Biochem1974; 47: 469-474.
27. Mohandas J, Marshall JJ, Duggin GG, Hovath JS and Tiller DJ: Differential distribution of glutathione and glutathione related enzymes in rabbit kidney. Possible implications in analgesic nephropathy. Biochem Pharmacol1984; 33: 1801-1807.
28. Santos MT, Valles J, Aznar J and Vilches J: Determination of plasma malondialdehyde-like material and its clinical application in stroke patients. J Clin Pathol1980; 33: 973-976.
29. Sambrook J, Fritsch EF and Maniatis T: Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, New York, Second Edition, 1989.
30. Birren B, Lai E: Pulsed Field Gel Electrophoresis: A Practical Guide. Academic Press, San Diego, 1993.
31. Adam M, Fietzek P and Kuhn K: Investigations on the reaction of metals with collagen in vivo. 2. The formation of cross-links in the collagen of lathyritic rats after gold treatment *in vivo*. Eur J Biochem1968; 3: 411-414.
32. Harborne JB, Williams CA: Flavone and Flavonol Glycosides: In The Flavonides. Harborne JB, Mabry TJ and Mabry H (Eds), Chapman and Hall, London, 1975.
33. Gohari AR, Saeidnia S, Shahverdiar AR, Yassa N, Malmir M, Molllazade K and Naghinejad AR: Phytochemistry and antimicrobial compounds of *Hymenocrater calycinus*. Eurasain JBiosci2009; 3: 64-68.
34. El-mousallamy AMD, Hawas UW and Husein SAM: Teucrol, a decarboxyrosemerinic acid and its 4'-O-triglycoside, tuecroside from *Teucrium pilosum*. Phytochemistry2000; 55: 927-931.
35. Ali M: Techniques in Terpenoids Identification. Birla Publications, Delhi, India, 2001.
36. Priya KS, Gnanamani A, Radhakrishnan N and Babu M: Healing potential of *Datura alba* on wounds in albino rats. J Ethanopharmacol 2002; 83: 193-199.
37. Ehrlich HP, Hunt TK: Collagen Organization Critical Role in Wound Contraction. Adv Wound Care2012; 1: 1-9.
38. Chitra P, Ajit S and Indira CP: Evaluation of wound healing activity of hydroalcoholic extract of leaves of *Stachytarpheta jamaicensis* in streptozotocin induced diabetic rats.Der Pharmacia Lettre 2013; 5: 193-200.
39. Agarwal PK, Singh A, Gaurav K, Goel S, Khanna HD and Goel RK: Evaluation of wound healing activity of extracts of plantain banana (*Musa sapientum* var. *paradisiaca*) in rats. Ind J Exp Biol 2009; 47: 32-40.
40. Gutierrez RMP, Solis RV: Anti-inflammatory and wound healing potential of *Prosthechea michuacana* in rats. PharmacogMag2009; 5: 219-225.
41. Hu M, Sebelman EE, Cao Y, Chang J and Hentz VR: Three dimensional hyaluronic acid grafts promote wound healing and reduce scar formation in skin incision wounds. J Biomed Mater Res B Appl Biomater 2003; 67: 586-592.
42. Nithya M, Suguna L and Rose C: The effect of nerve growth factor on the early responses during the process of wound healing. Biochem Biophys Acta 2003; 1620: 25-31.
43. Tomasek JJ, Gabbiani G, Hinz B, Chaponnier C and Brown RA: Myofibroblasts and mechanoregulation of connective tissue remodelling. Nat. Rev. Mol Cell Biol 2002; 3: 349-346.
44. Prashanthi R, Mohan N and Siva GV: Wound healing property of aqueous extract of seed and outer layer of *Momordica charantia* L. on albino rats.Indian JSci Technol2012;5:1936-1940.
45. Panchatcharam M, Miriyala S, Gayathri VS and Suguna L: Curcumin improves wound healing by modulating collagen and decreasing reactive oxygen species. Mol and Cell Biochem2006; 290: 87-96.
46. Gupta A, Singh RL and Raghubir R: Antioxidant status during cutaneous wound healing in immunocompromised rats. Mol and Cell Biochem 2002; 241: 1-7.
47. Russo A, Longo R and Vanella A: Antioxidant activity of propolis: role of caffeic acid phenethyl ester and galangin. Fitoterapia2002; 73: 21-29.

48. Choi BS, Song HS, Kim HR, Park TW Kim TD, Cho BJ, Kim CJ and Sim SS: Effect of coenzyme Q10 on cutaneous healing in skin-incised mice. Arch Pharm Res2009;32: 907-913.

How to cite this article:
Rais I and Md. Ali: Phytochemical Investigation for the Wound Healing Potential of A Novel Compound Isolated From *Mentha Piperita* L. Leaves. Int J Pharm Sci Res 2016; 7(7): 2781-94.doi: 10.13040/IJPSR.0975-8232.7(7).2781-94.