Phytochemical composition of *Plectranthus tenuiflorus* extract and study some of its medical applications

By:

Tagreed Alsufyani[1], Asif fatani[2], Suad Shaker[3], Faten Korshid[3], Hassan albar[1]

[1]Chem. Dept., Email: Halbar@kau.edu.sa. [2]King Abdulaziz University Hospital, Clinical Microbiology Lab, [3]King Fahad Medical Research Center, P.O. Box 9028, Jeddah21589, Saudi Arabia.

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1Chem. Dept., Email: Halbar@kau.edu.sa. 2King Abdulaziz University Hospital, Clinical Microbiology Lab, 3King Fahad Medical Research Center, P.O. Box 9028, Jeddah21589, Saudi Arabia.

Abstract

The fresh leaf of *Plectranthus tenuiflorus* (Lamiaceae) was collected from Taif in Saudi Arabia and brought under quantitative and qualitative estimation for metallic elements. The fresh leaf was crushed to analyze the obtained juice for some chemical constituents by way of phytochemistry. The findings revealed the existence of whole carbohydrates at a concentration of 5.98 × 10-5 M of total leaf components. Paper chromatography separation proved that leaf contain 7 protein amino acids represented by Ala; His; Phe; Asn; Asp; Glu and Leu. The descriptive tests showed the presence of coumarins, hydrolysable tannins, essential oil, being thymol (62.53%) the major component in the oil and triterpenoids and in the contrast the absence of alkaloids, steroids, anthraquinones, flavonoides, condensed tannins, cardiac- and anthraquinone glycosides. These phytochemical results were compared with other of *Euryops arabicus* (Soam) and *Clutia myricoides* (Soa'bor).

The study evaluated in vitro antimicrobial and fibroblast proliferation activities of this plant material with in vivo study of its efficiency on enhancing wound healing process in Wister rats. The juice showed inhibitory effect limited to growth of *S. pyogenes* (17.8 mm) and *P. aeruginosa* (17 mm) by agar diffusion method. The percentage of *P. aeruginosa* radial growth inhibition under leaf juice effect indicated medial activity of this plant material.

The juice caused a significant dose- and time-related catalyzing of fibroblasts proliferation (0.1% w/v after 72 hrs), IC50 appeared after 24 hrs at 0.5% (w/v) of the juice.

The results clearly substantiate the beneficial effects of *P. tenuiflorus* juice in accelerating wounds healing at 10% (w/v) concentration through daily and topical application to the wound area compared to the control group and other concentrations and extracts used in earlier studies, where the healing process took 14 days with appearance of hair follicles and sebaceous glands at the whole wound area, including the scar, and looking very close the way it is in normal skin. Consequently, this plant is a promising source of a natural wound healer.

References

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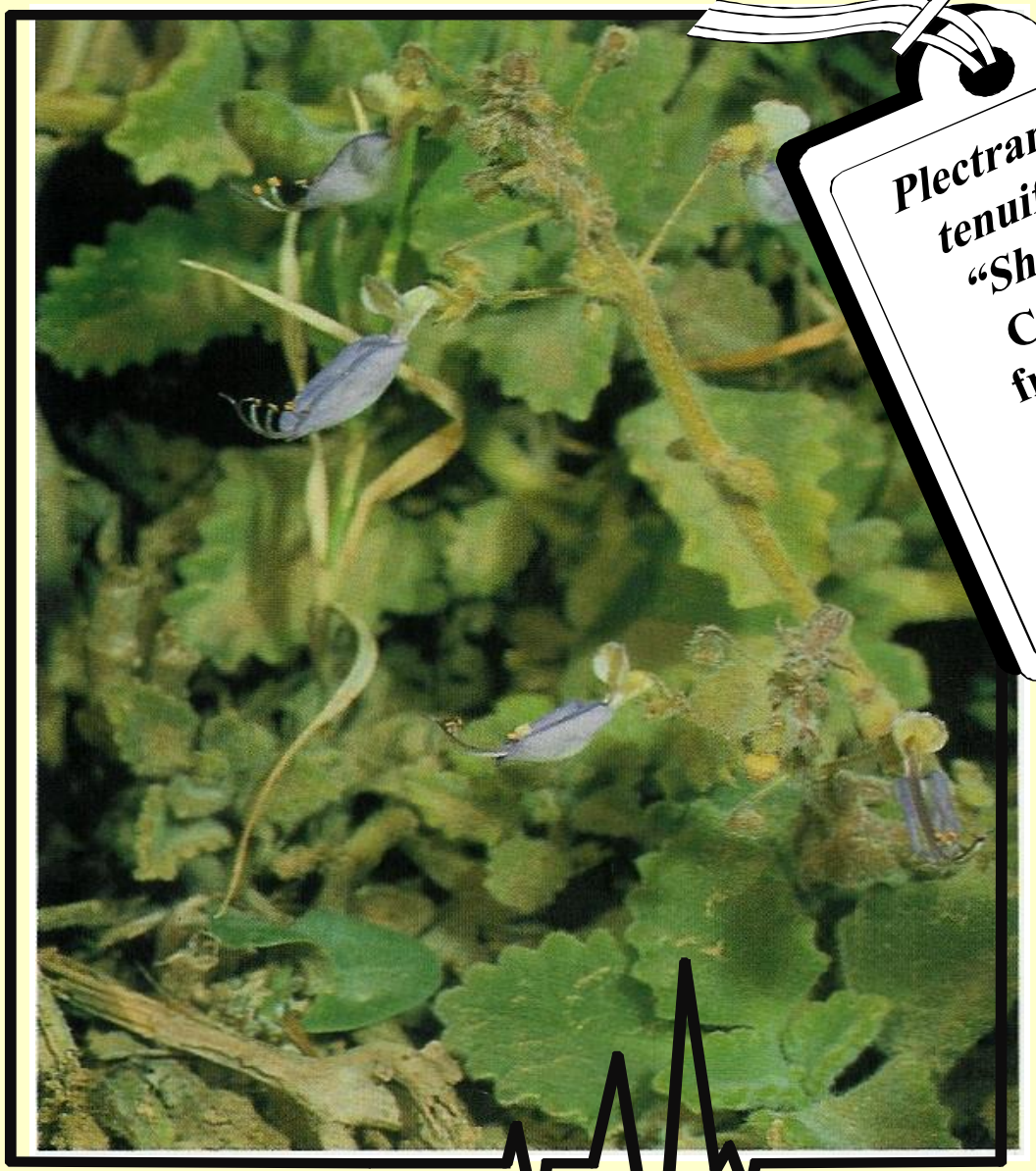
[2] Albar H. A., Abdel-Mogib M. and Batterjee S. M., “Chemistry of the Genus *Plectranthu”,*. ***Molecules*** *7*: 271-301, 2002.
[3] Albar H. A., Alsufyani T. and Soliman M., Unpublished results (2006).



***Plectranthus tenuiflorus* "Sharah"**
Collected from Al-sir valley on 1700 m height from sea surface

Metallic Elements Estimation In Leaf By ICP-OES

Metals	Concentration (ppm)
Ag	0.00033 ± 0.001 i
Al	9.15933 ± 0.19 g
As	0
Ba	0.23467 ± 0.07 i
Bi	0
Ca	903.16333 ± 0.21 a
Cd	0
Co	0.025 ± 0.002 i
Cr	0.05567± 0.01 i
Cu	0.09333 ± 0.05 i
Fe	30.80333 ± 0.23 d
Mg	367.09333 ± 0.18 b
Hg	0

Metals	Concentration (ppm)
Mn	1.02233 ± 0.22 h
Mo	0.01933 ± 0.01 i
Na	75.07333 ± 0. 17 c
Ni	0.08833 ± 0.03 i
P	24.87 ± 0.3 e
Pb	0.09167 ± 0.07 i
Sb	0.03433 ± 0.03 i
Se	0
Sr	12.35333 ± 0.97 f
V	0.23167 ± 0.001 i
Zn	0.37933 ± 0.05 i

Data in the column followed by different letters are significantly different at $p \leq 0.05$ according to LSD test.

Primary metabolisms

Protein Amino Acids detected by using paper chromatography

Ala, Leu, Glu, Asp, Asn, Phe & His.

Total Carbohydrates determined according to Allen *et al.* method

Statistical study of Least square method was used

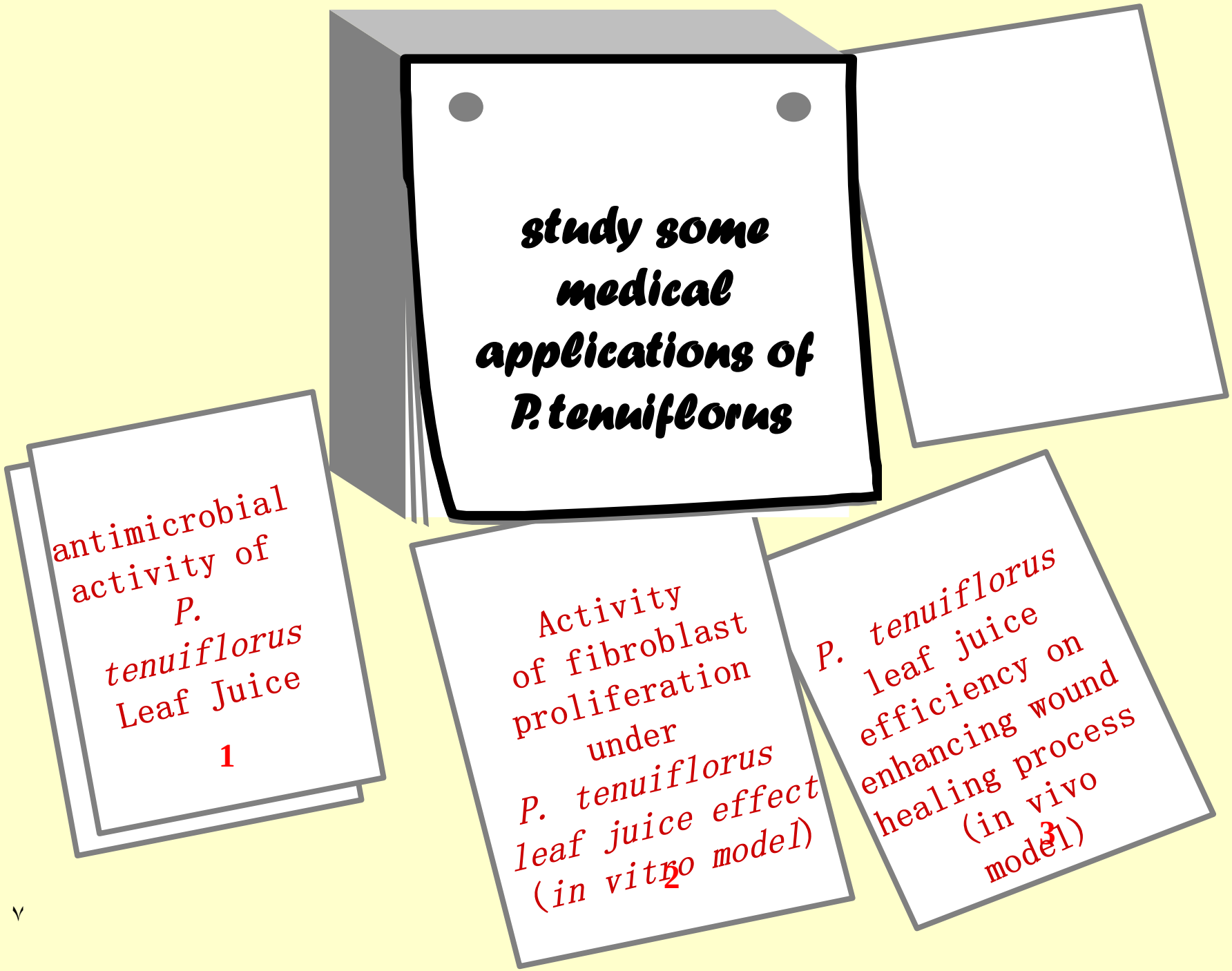
5.98×10^{-5} M

Secondary metabolisms

		Plectranthus tenuiflorus	*Euryops arabicus*	*Clutia myricoides*
Glycosides	Anthraquinon		+	+
	Cardiac	-	+	+
	Saponins	-	+	+
Flavonides	Anthraquinons	-	+	+
	Flavonides	-	+	+
	Coumarins	+	+	+
	Tannins	Hydrolysable tannins	Condensed tannins	Condensed tannins
Isoprenoides	Essential oils	+	+	-
	Triterpenoids	+	+	+
	Steroids	-	+	+
Nitrogenous compounds	Alkaloids	-	+	+
	Quaternary Alkaloids	-	+	-

+ indicates that product is existing.

- indicates that product is not existing.


study some medical applications of P.tenuiflorus
antimicrobial activity of P. tenuiflorus Leaf Juice
1
Activity of fibroblast proliferation under P. tenuiflorus leaf juice effect (in vitro model)
2
P. tenuiflorus leaf juice efficiency on enhancing wound healing process (in vivo model)
3

1

Antimicrobial Activity of *P. tenuiflorus* Leaf Juice by Agar-well diffusion

Pathogenic microbes	Means of Inhibition Zone (mm)[1] ± SD under effect of			
	Leaf juice of *P. tenuiflorus*	Penicillin (10unit)	Gentamicin (10µg)	Nystatin (25µl)
Streptococcus pyogenes	17.8 ± 0.36[a]	40 ± 0.02	nt	nt
Staphylococcus aureus	-	32 ± 0.01	nt	nt
Pseudomonas aeruginosa	17 ± 0.35 [b]	nt	15.5 ± 0.01	nt
Klebsiella pneumoniae	-	nt	20 ± 0.01	nt
Escherichia coli	-	nt	19 ± 0.00	nt
Candida albicans	-	nt	nt	12 ± 0.01

Data in the first column followed by different letters are significantly different at $p \leq 0.05$ according to independent sample t-test.

(-), inactive; nt, not tested.

[1] Diameter of inhibition zone (mm) including well diameter of 4 mm.

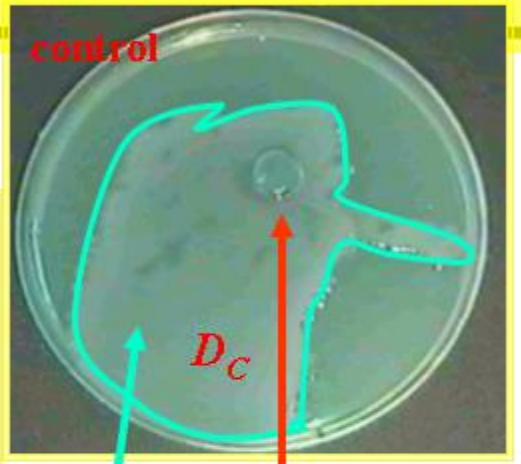
1

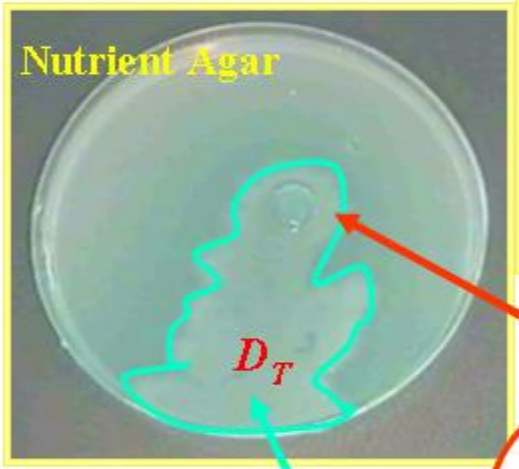
Percentage Radial Growth Inhibition of *Pseudomonas aeruginosa* under effect of *P. tenuiflorus* Leaf Juice on Nutrient and Muller-Hinton Agar.

Percentage growth inhibition =

$$\frac{D_C - D_T}{D_C} \times 100$$

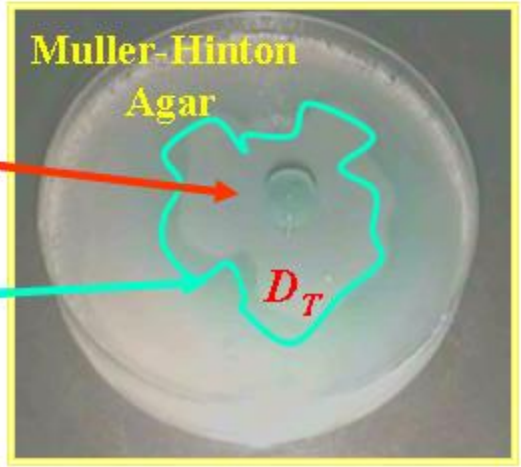
Were: D_C = is the average diameter of *P. aeruginosa* growth with control. D_T = the average diameter of *P. aeruginosa* growth with treatment.





The significant differences between radial growth of *P. aeruginosa* on NA and MHA under *P. tenuiflorus* leaf juice was found by Independent sample t-test at α=.05.

10mm diameter of 0.5 McFarland of *P. aeruginosa* mass



Radial growth of *P. aeruginosa*

② fibroblast proliferation activity under effect of *P. tenuiflorus* leaf juice (*in vitro model*)

Were the initial density of fibroblast proliferation/ mL = 1 × 10^5 cell/ mL

			the means of fibroblast density × 10^5/ mL ± SD		
			after 24 h	after 48 h	after 72 h
Minimum Essential Medium with:	2% FCS Control		1.5 ± 0.1 a 3	2.05 ± 0.05 b 2	2.65 ± 0.04 b 1
	Deferent concentration of leaf juice	0.05%	1.15± 0.05 b 3	2.35 ± 0.04 a 2	2.7 ± 0.1 b 1
		0.1%	1.5 ± 0.02 a 3	2.3 ± 0.1 a 2	3.15 ± 0.06 a 1
		0.3%	1.1 ± 0.2 b 2	1.9 ± 0.2 b 1	1.4 ± 0.1 c 2
		0.5%	0.75 ± 0.04 c 1	0.75 ± 0.04 c 1	0.05 ± 0.04 d 2
		1%	0.2 ± 0.03 d 1	0 ± 0.001 d 2	0 d 2

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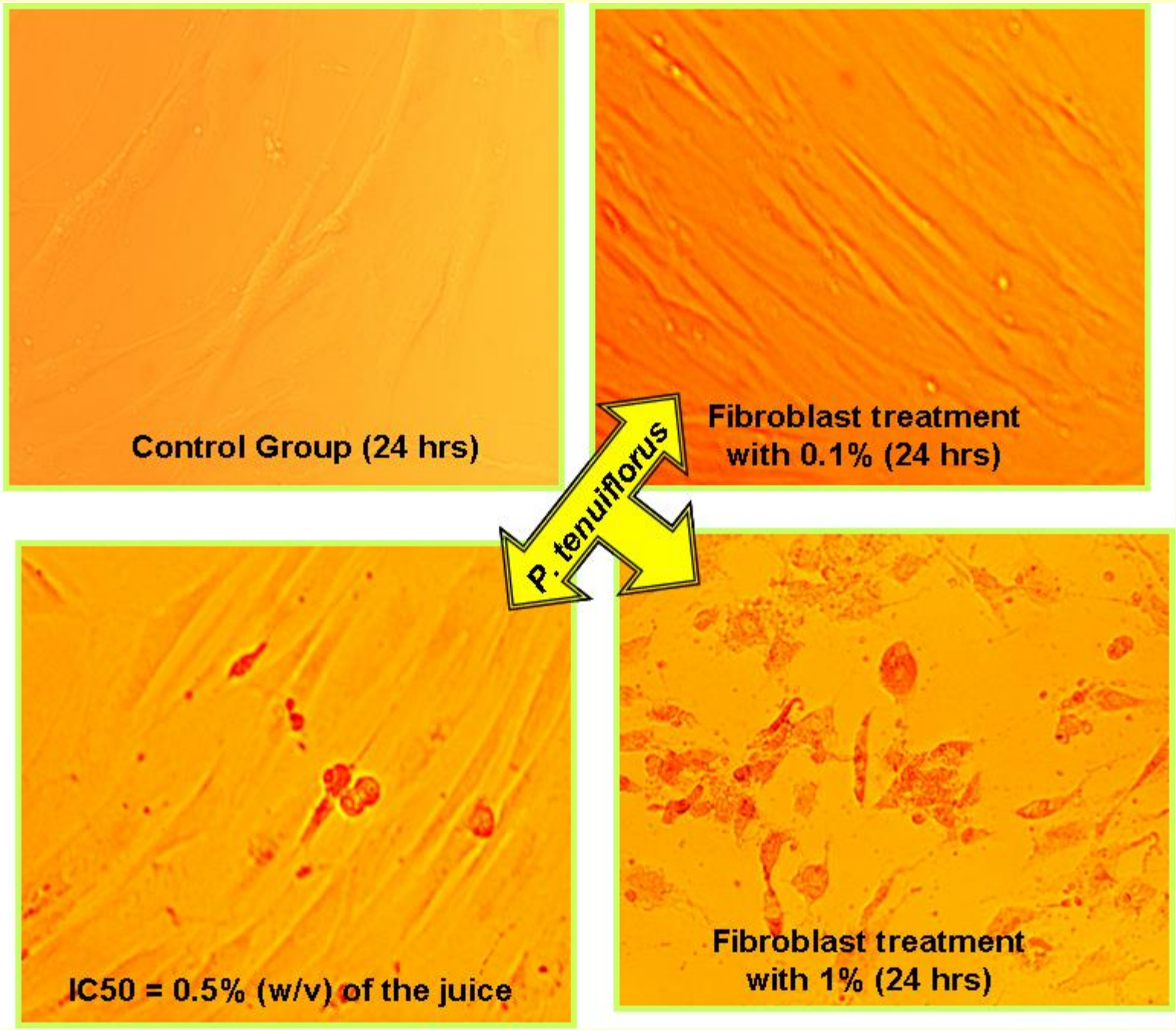
Data in the raw followed by different numbers are significantly different at $p \leq 0.05$ according to LSD test.


Control Group (24 hrs)
Fibroblast treatment with 0.1% (24 hrs)
P. tenuiflorus
IC50 = 0.5% (w/v) of the juice
Fibroblast treatment with 1% (24 hrs)

③ *P. tenuiflorus* leaf juice efficiency on enhancing wound healing process (*in vivo model*)

		The mean of wound contraction rate(%) ± SD				
		4th day	8th day	12th day	14th day	16th day
Control group		38.96 ± 1.14 c	67.58 ± 0.32 c	83.55 ± 0.84 c	87.94 ± 0.41 b	93.75 ± 0.84
Group treated with *P. tenuiflorus*	80%	50.14 ± 0.47 b	80.92 ± 0.37 b	96.37 ± 0.21 b	99 ± 0.11 a	Complete healing
	10%	53.29 ± 0.86 a	88.1 ± 0.27 a	99.25 ± 0.12 a	Complete healing	

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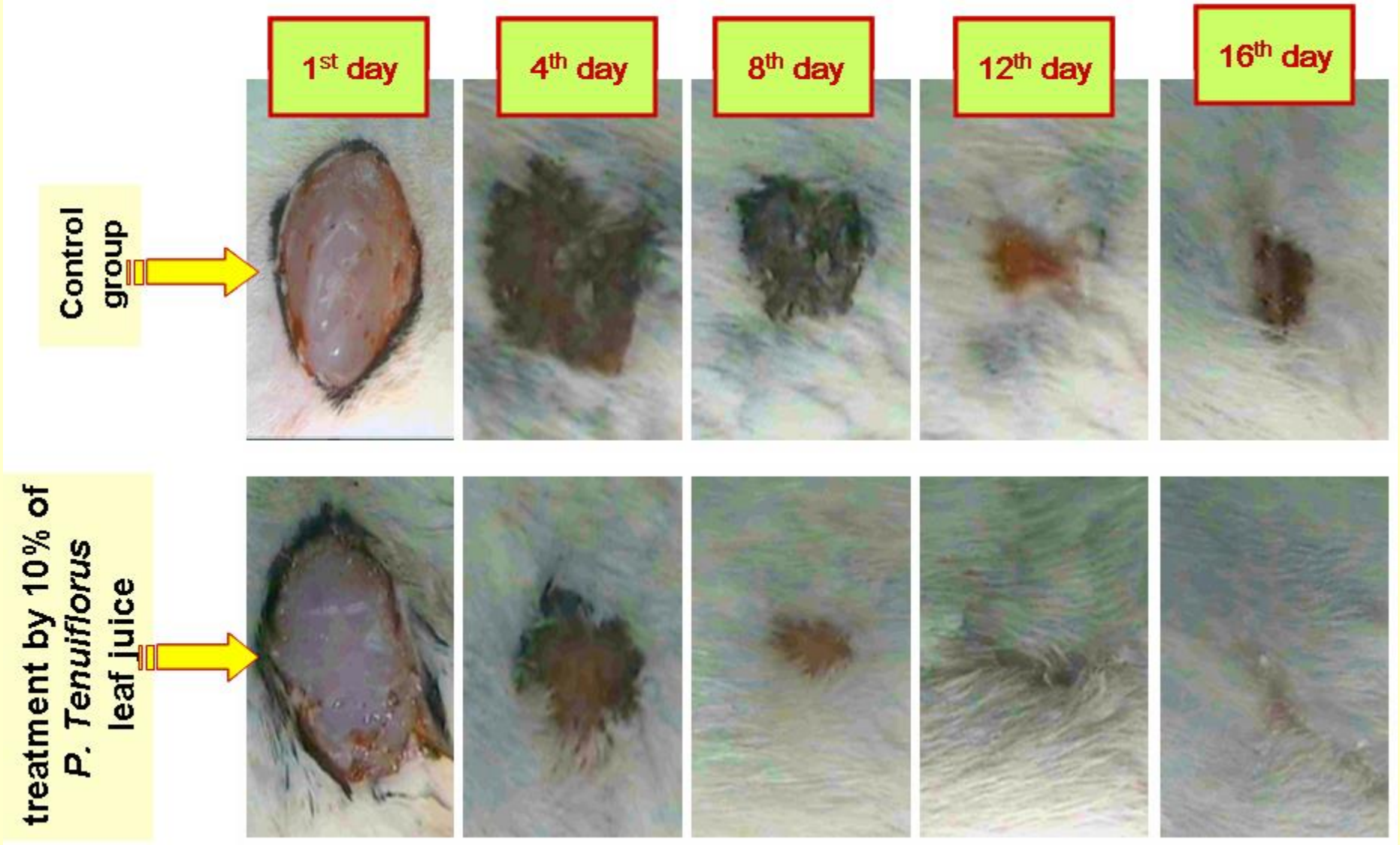
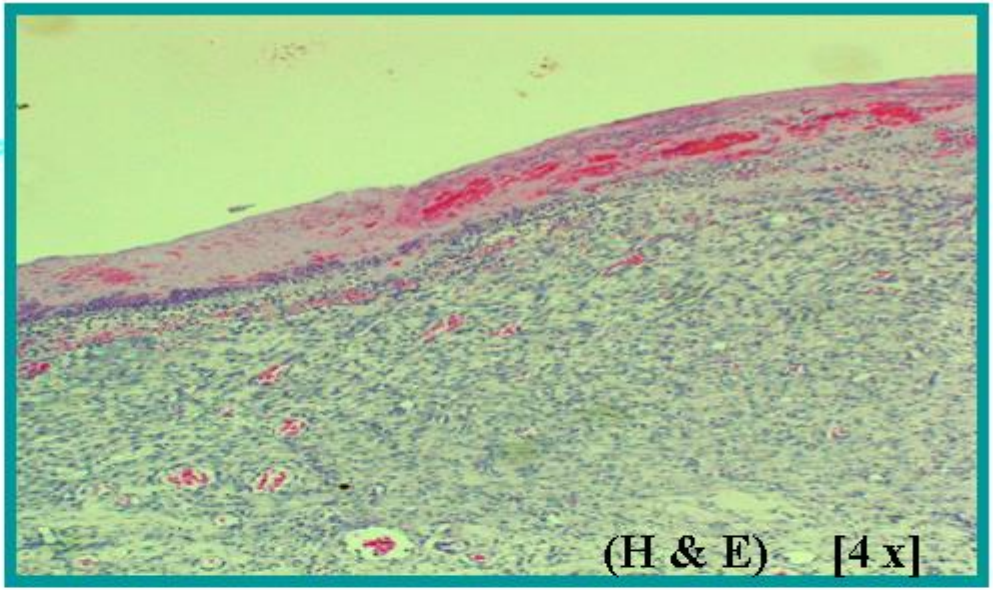
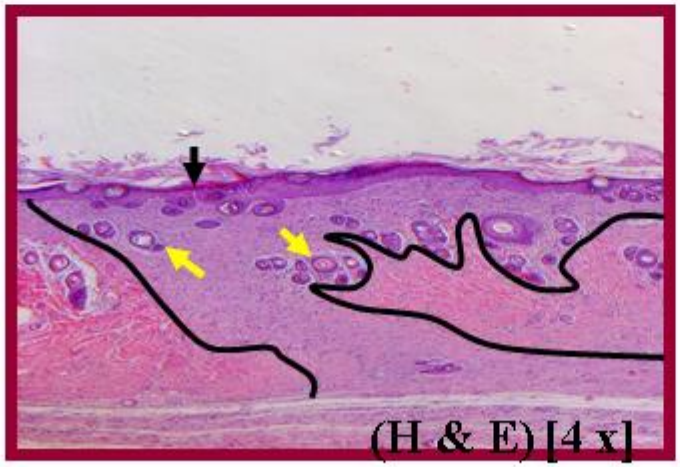
Photography of wound healing process
(*in vivo model*)

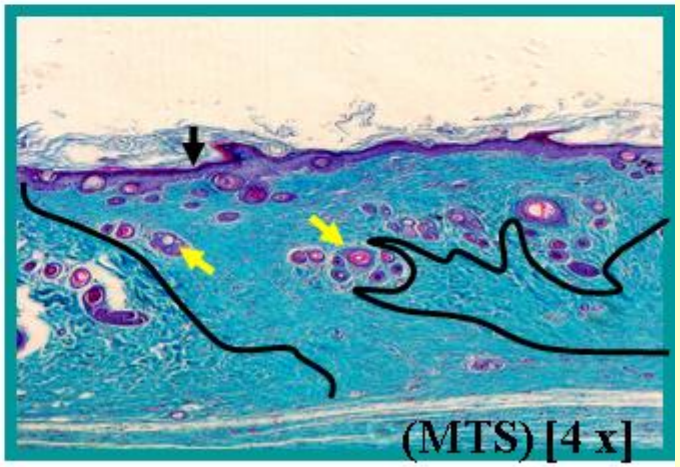


Histic studies of wound area at 14th day



Untreated wound at 16 days Lacking epitheization





Healthy scar with complete epithelization → reappearance of skin appendages (hair follicles and glands) →