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EFFECT OF CERTAIN HERBAL EXTRACT(S) ON PROMOTION OF SKIN WOUND HEALING IN RATS

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Abstract

Wound healing is a complicated process involving different tissues and cell lineages. Herbal medicines based on plant extracts have been used to accelerate the wound healing process since ancient time. Therefore, the aim of our study was to investigate the potential wound healing activity of two herbal extracts: *Tecomaria capensis* and green tea leaves formulated as ointments in non-infected and infected excision wounded rats, and to determine the possible underling mechanisms.

Phytochemical examination of methanolic extracts of *Tecomaria capensis* and green tea leaves revealed that *Tecomaria capensis* has higher phenolic and flavonoid content than green tea. Moreover, both extracts have relatively equal in antioxidant activities. The wound healing potential of *Tecomaria capensis* and green tea ointment extracts were determined in two concentrations (0.6% & 6% in Vaseline base) for topical treatment of non-infected and infected excision wounds in rats. Treatments were applied for 14 days for non-infected and 16 days for infected models. *Tecomaria capensis* and green tea ointment extracts, in both concentrations, caused a significant increase in wound contraction rate and glutathione level, as well as a significant decrease in lipid peroxidation and total leukocytic count. Also, both herbal extracts significantly reversed the elevated level of transforming growth factor-beta (TGF-β), tumor necrosis factor-alpha (TNF-α) and vascular endothelial growth factor (VEGF) caused by the excision wounds. Moreover, histological examination showed a marked improvement in the skin condition.

This study concludes that *Tecomaria capensis* and green tea extracts were effective in enhancing the healing process in both non-infected and infected excision wounds due to their antioxidant, anti-inflammtory and antiangiogenic effects. This could be attributed to their high phenolic and flavonoid contents.

Key words: *Tecomaria capensis*; Green tea; Wound healing; Oxidative stress; Inflammation.

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List of Abbreviations

ADP	Adenosine diphosphate
ATP	Adenosine triphosphate
BCB	Beta carotene bleaching assay
b-FGF	Basic fibroblast growth factor
BHA	Butylated hydroxyl anisol
BW	Body weight
CCl_4	Chloroform
CFU	Colony forming unit
DNA	Deoxyribonucleic acid
DPPH	2,2′-diphenyl-1-picrylhydrazyl
EC	Epicatechin
ECG	Epicatechin gallate
ECM	Extracellular matrix
EGC	Epigallocatechin
EGCG	Epigallocatechin-3-gallate
EGF	Epidermal growth factor
FCR	Folin-ciocalteau reagent
FGF	Fibroblast growth factor
GSH	Reduced glutathione
GT	Green tea
H&E	Hematoxylin and eosin stain
H_2O_2	Hydrogen peroxide
HIF-1*α*	Hypoxia-inducible factor-1alpha
IL-1	Interleukin-1
IL-6	Inteleukin-6
IL-8	Interleukine-8
MDA	Malondialdehyde
MMPs	Matrix metalloproteinase
MRSA	Methicillin resistant *staphylococcus aureus*
MSSA	Methicillin-sensitive *staphylococcus aureus*
NADPH	Nicotinamide adenine dinucleotide phosphate reduced
NODCAR	National organization for drug control and research
$O•_2$	Superoxide anion
P. aeruginosa	*Pseudomonas aeruginosa*
PDGF	Platelet-derived growth factor

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RNS	Reactive nitrogen species
ROS	Reactive oxygen species
S. aureus	*Staphylococcus aureus*
SCVs	Small-colony variants
SFD	Staphylococcal foodborne diseases
SOD	Superoxide dismutase
TBA	Thiobarbituric acid
TBARS	Thiobarbituric acid reactive substances
TBHQ	*Tert*-butylated hydroxyl qunione
TC	*Tecomaria capensis*
TGFR	Transforming growth factor receptor
TGF-β	Transforming growth factor-beta
TNF-α	Tumor necrosis factor-alpha
UV	Ultra violet
VEGF	Vascular endothelial growth factor
α-SMA	Alpha-smooth muscle actin

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Introduction

The skin

Skin is the outermost tissue of the human body, the interface between humans and their environment and the largest organ in terms of both weight and surface area. It has an area of approximately 16, 000 cm^2 for an adult and represents about 15% of the body weight **(Morkhande *et al*., 2016)**.

Skin anatomy

Skin is a dynamic organ in a constant state of change, as cells of the outer layers are continuously shed and replaced by inner cells moving up to the surface. Although the skin is structurally consistent throughout the body, skin varies in thickness according to anatomical site and age of the individual **(Abd *et al*., 2016)**. There are three structural layers to the skin: the epidermis, the dermis and subcutaneous tissue **(Kanitakis, 2002)**. The epidermis is the outer layer, serving as the physical and chemical barrier between the interior body and exterior environment; the dermis is the deeper layer providing the structural support of the skin, below which is a loose connective tissue layer, the subcutaneous tissue or hypodermis which is an important depot of fat. Hair, nails, sebaceous, sweat and apocrine glands are regarded as derivatives of skin **(Morkhande *et al*., 2016)** (Fig. I).

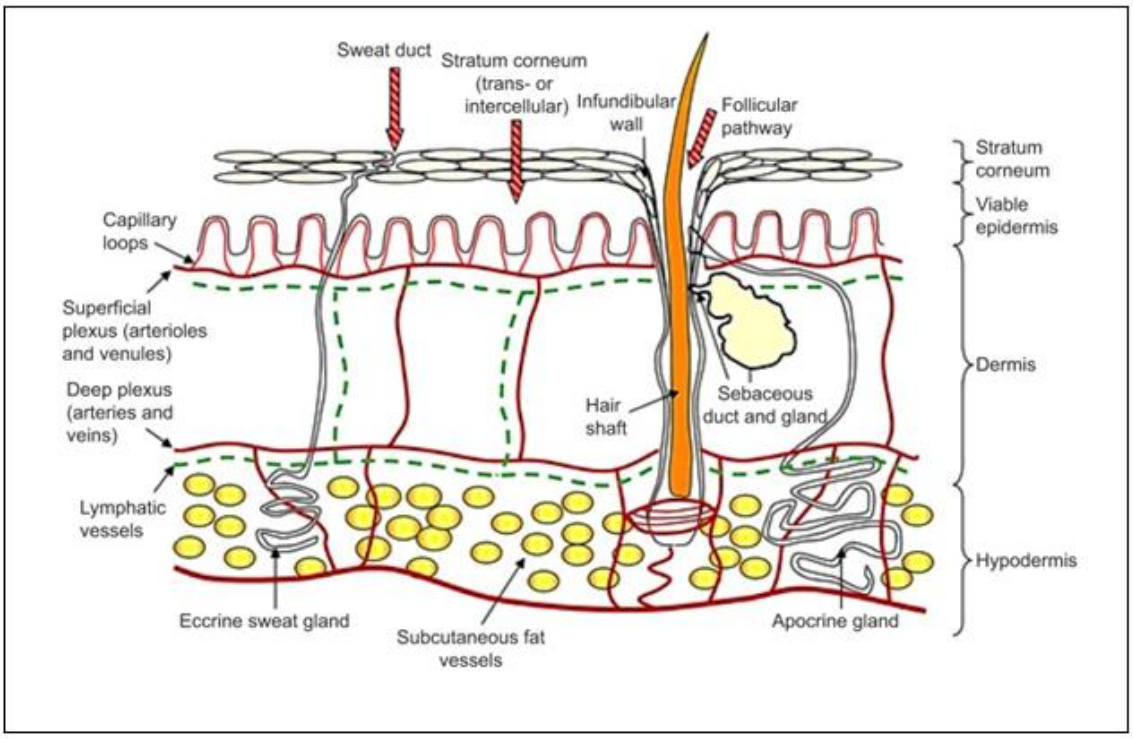

Fig. (I). Structure of the skin (Abd *et al*., 2016).

Skin functions

1. Protection: The skin serves as the main protective barrier, preventing damage to internal tissues from physical trauma, ultraviolet (UV) light, temperature changes, toxins and bacteria **(Butcher & White, 2005)**. As well as preventing harmful substances from entering the body, it also controls the loss of vital substances.

2. Sensation: The nerve endings present in the skin allow the body to detect pain, and changes in temperature, touch and pressure **(Lee & Caterina, 2005)**.

3. Thermoregulation: allowing the body to respond to changes in temperature by constricting or dilating the blood vessels within it **(Timmons, 2006)**. The sweat glands produce sweat which stays on the skin allowing the body to cool down. The skin provides a relatively dry and semi-impermeable barrier to fluid loss **(Madison, 2003)**.

4. Excretory function: The skin excretes waste products in sweat which contains water, urea and albumin. Sebum is an oily substance which is excreted by the sebaceous glands, helping to lubricate and protect the skin **(Lee & Caterina, 2005)**.

5. Metabolism: When UV light is present, the skin makes activation for vitamin D which is required for calcium absorption **(Bikle, 2014)**.

6. Absorption: Medicine can be administered through the skin, by ointments, creams or by means of adhesive patch, such as the nicotine patch or iontophoresis **(Alexander *et al.*, 2012)**.

The skin can allow non-verbal communications that convey changes in mood through color changes such as blushing. The skin also gives clues as to our physical well-being **(Jack & Schyns, 2015)**.

The skin needs to remain intact to allow the body to perform these vital functions. When the skin is breached, it is important to close the defect as quickly as possible, thereby preventing infection from occurring and allowing normal skin function to return.

Wounds

Wound is described as physical injury that occurs as result of an opening or break of the skin **(Agra *et al*., 2013).**

Classification of wounds

1. According to chronicity

Wounds could be classified as acute or chronic. An acute wound is an injury to the skin that occurs suddenly rather than over time. It heals at a predictable and expected rate according to the normal wound healing process. A chronic wound develops when any acute wound fails to heal in the expected time frame for that type of wound, which might be a couple of weeks or up e.g. ulcer, burn wound **(Phillips, 2001)**.

2. According to affected layers

Wounds may also be classified according to the number of affected layers. When damage is limited to the epithelial tissue (epidermis) alone, wound is regarded as a superficial and usually heals rapidly by regeneration of epithelial cells. Wound with a partial thickness involves the deeper dermal layer and includes vessel damage, whereas that of a full thickness affects the subcutaneous fat layer and beyond. Healing of the wound of full thickness requires the synthesis of new connective tissue and takes longer time **(Mast, 1992; Paul & Sharma, 2004)**.

Wound healing

Cutaneous wound healing represents a morphogenetic response to injury that provides adequate tissue perfusion, oxygenation, proper nutrition and moist wound healing environment for restoration of disrupted anatomical continuity and disturbed functional status of the affected part **(Begum & Nath, 2000)**.