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Review article

Assessment of non-invasive techniques and herbal-based products on dermatological physiology and intercellular lipid properties

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ABSTRACT

Skin is the largest external organ of the human body. It acts as a barrier to protect the human body from environmental pollution, mechanical stress, and excessive water loss. The defensive function resides primarily on top of the epidermis layer commonly known as stratum corneum (SC). Human SC consists of three major lipids, namely ceramide, free fatty acid, and cholesterol that comprise approximately 50%, 25%, and 25% of the total lipid mass, respectively. The optimal composition of SC lipids is the vital epidermal barrier function of the skin. On the other hand, skin barrier serves to limit passive water loss from the body, reduces chemical absorption from the environment, and prevents microbial infection. In contrast, epidermal lipids are important to maintain the cell structure, growth and differentiation, cohesion and desquamation as well as formation of a permeability barrier. Multiple non-invasive *in vivo* approaches were implemented on a regular basis to monitor skin physiological and intercellular lipid properties. The measurement of different parameters such as transepidermal water loss (TEWL), hydration level, skin elasticity, collagen intensity, melanin content, sebum, pH, and tape stripping is essential to evaluate the epidermal barrier function. Novel non-invasive techniques such as tape stripping, ultrasound imaging, and laser confocal microscopy offer higher possibility of accurate and detailed characterisation of skin barrier. To date, these techniques have also been widely used to determine the effects of herbal plants in dermatology. Herbal plants have been traditionally used for ages to treat a variety of skin diseases, as reported by the World Health Organisation (WHO). Their availability, lower cost, and minimal or no side effects have created awareness among society, thus increase the demand for natural sources as the remedy to treat various skin diseases. This paper reviews several non-invasive techniques and evaluations of herbal-based product in dermatology.

1. Introduction

Non-invasive procedures can be defined as treatment without incision into the skin and contact with a mucous membrane or internal body cavity other than through a natural or artificial body orifice. The procedures and instruments are classified as safe and simple. The term "non-invasive" can be translated as "no harm", "no contact", "no alteration of structure or function", and "maintaining integrity of organism" [1]. Therefore, non-invasive can be literally interpreted as "a procedure or instrument that causes minimal and temporary changes to structure or function, such as painless, without incision or blood loss" [1]. Nowadays, the non-invasive techniques have great benefits and huge capabilities in determining the skin's physiological and intercellular properties especially for characterization of skin barrier.

Skin intercellular lipid characterises and determines the profiling of lipid species in the biological system which is highly associated with skin type. Skin barrier mainly comprises of corneocytes and a lipid-enriched intercellular matrix. Ceramide is the major lipid found in SC, with 50% abundancy, followed by free fatty acid (25%) and cholesterol (25%) [2]. These extracellular lipids are secreted from lamellar bodies (LB) into the intercellular space of SC. LB contain phospholipids, spingomyelin, glucosylceramide, and cholesterol, which are metabolised by enzymes and secreted into intercellular lipids [3].

The epidermal barrier plays an important role in the development of atopic eczema (AE), while skin lipids contribute to barrier integrity. It

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limits passive water loss, reduces chemical absorption from the environment, and prevents microbial infection. The defensive function resides primarily in the upper part of the epidermis, whereby the skin barrier is integrated with the SC formation and homeostasis. For example, the decrease of SC hydration and permeability alteration of barrier functions could lead to various skin disorders, such as atopic dermatitis, psoariasis, and ichthyosis [4, 5, 6]. That being said, a proper development and maintenance of SC is the key to its remarkable ability to defend the body against both chemical and microbial attacks as well as dehydration [7].

Non-invasive techniques have been established for centuries to determine the herbal plant efficacy in treating skin diseases. Nowadays, the application of herbal products has become a natural approach to a healthy lifestyle [8]. The fact that natural remedies are more reliable and efficient to treat skin diseases with minimal or no side effects as compared to other conventional drugs draw massive attention in dermatology study [9, 10, 11]. Owing to the increasing cost of maintaining personal health, natural remedies have become more common to treat minor ailments [12], thus becomes the major reason for the increasing demands for natural-based remedy in Asia, especially China (Wu-Hsing), India (Aryuvedic, Unani, Siddha), and Japan (Kampo) [13, 14, 15].

Herbs have been classified as potential agricultural commodities under the National Key Economic Area (NKEA) and are expected to contribute to the country's income and create employment opportunities. For example, Mas Cotek (*Ficus deltoidea*), Misai Kucing (*Ortho siphon aristatus/stamineus benth*), Lidah Buaya (*Aloe Vera Inn*), and Tongkat Ali (*Eurycoma longifolia*) are potential herbal crops for medicinal use. In Malaysia, the herbal industry has a great potential to encourage the national tourism and business development, especially in pharmaceutical and cosmeceutical industries [8].

However, herbal medicinal must fulfil the technical safety and application standards (norms) required by society. Both local and international products are regulated under Sale of Drugs Act 1952 (Revised 1989) and Control of Drugs and Cosmetics Regulations 1984 (amended 2009). This paper focused on non-invasive techniques to assess skin physiology and intercellular lipid properties which is correlated to skin physiological conditions and epidermal lipid profiles. The efficacy of herbal plants using the non-invasive techniques to treat skin diseases was briefly discussed. Literature was obtained from the following database: Science Direct, PubMed, Google Scholar, and Springer Link for scientific publications.

2. Epidermal skin structure

The epidermis is the outermost layer of the skin. In general, epidermis consists of basal layer (source of replacement cell), spinous layer (centre of the epidermis where keratinocytes make keratin), granular cell layer (site of water barrier), and stratum corneum (thick keratinised outer layer which prevents water loss and provides anti-trauma and anti-infectious barrier). It is made up of 95% keratinocytes, Langerhans cells, Merkel cells, inflammatory cells as well as melanocytes. Melanocytes (found in the basal layer) are specialised neural crest cells which produce melanin, a protective pigment that absorbs harmful UV radiations and produces energy as harmless heat through a route referred to as ‘ultrafast internal’. Langerhans cells are the immune cells responsible in antigen presentation, which literally assist the skin's immune system. Merkel cells found in the basal layer are associated with sensory nerve endings [16].

The skin is responsible to guard underlying muscles, bones, ligaments, and internal organs. There are two general types of skin, namely hairy and glabrous skin [17]. However, the skin can be dry, sensitive, pale, sagging, or tired. Individuals who suffer from beta-carotene, B complex, vitamins C and E deficiency often encounter dry skin problems. Considering the fact that skin interfaces with the environment, skin plays a key role in protecting the body against pathogens [18, 19] and excessive water loss [19]. Besides, skin also plays an important role in insulation, temperature regulation, sensation, storage, the synthesis of vitamin D by the action of UV, the protection of vitamin B folates, the absorption of oxygen and drugs [20], and water resistance.

3. The non-invasive assessment of skin physiological conditions

The Multi Probe Adapter (MPA) system is used to measure skin biophysical properties. The modular system is a basic device equipped with specific digital probes that can be adjusted according to the user preferences. Calibration data are stored inside the system itself. The advantage of using this method is that the probes can be connected to any independent devices and simultaneously transmit data to related software. The probes can usually measure transepidermal water loss, hydration, melanin, erythema, elasticity, collagen, sebum, and pH of the skin.

3.1. Skin transepidermal water loss (TEWL)

Transepidermal water loss (TEWL) was measured regularly in order to provide further information on the epidermal permeability barrier—either normal, experimentally perturbed, or in diseased conditions [21]. Low TEWL values are a basic feature of in vivo intact skin function [21, 22]. Elevated TEWL values indicate the skin barrier abnormalities, which are the major reason of several diseases, such as atopic dermatitis and ichthyosis vulgaris [23, 24, 25, 26, 27, 28].

TEWL can be measured by evaporimeter (Tewameter® TM 300; Courage & Khazaka). Figure 1 illustrates the measurement principle, Tewameter® TM 300. Tewameter was particularly designed according to Nilsson's Vapour Pressure Gradient theory, with an open chamber method that provides minimal impact on the skin being examined with low statistical bias. The system consists of a hollow cylinder with two hygroscopic and temperature sensors to measure the density gradient of water evaporation pressure at different areas on the skin surface. The differences between the two measurements points are calculated by Fick's laws of diffusion in grams per hour per square meter ($g/h/m^2$), as stated in Eq. (1) [29]. However, the horny layer is not an inert membrane, but shows some affinity to water. Therefore, Fick's Law can be modified by the introduction of a partition coefficient Km [1]. Fick's law concept is a diffusive mass that will move from a region of high concentration to a region of low concentration across a concentration gradient.

$$\mathrm{Km} = \frac{(\text{Water concentration in the lower horny layer})}{(\text{Water concentration in the intercellular space of living epidermis})} \quad (1)$$

Fick's laws of diffusion suggest that the diffusion rate of gas across a permeable membrane can be evaluated by several factors, such as the chemical nature of the membrane, surface area, thickness, and partial pressure gradient of the gas (Table 1).

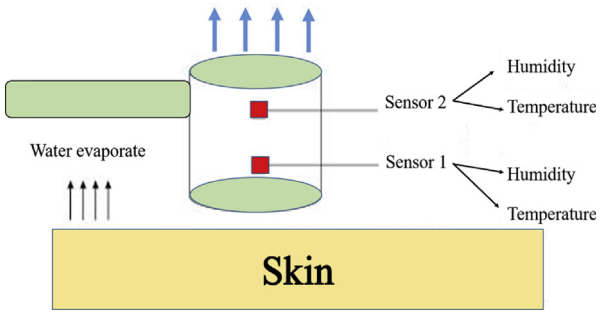

Figure 1. Illustration of the measurement principle, Tewameter TM 300 (Modified figure from) [30]).

Table 1. Interpretation of TEWL results [23].

TEWL – values g/h/m^2	Interpretation
0–10	Very healthy condition
10–15	Healthy condition
15–25	Normal condition
25–30	Strained skin
Above 30	Critical condition

3.2. Skin hydration

SC hydration is another important parameter that can be linked to assess epidermal functions. A variety of instrument- and environment-related variables, such as ambient air temperature, relative air humidity, and direct air flow may affect the hydration measurement. The factors originating from an individual include the age, sex, anatomic site, sweat, and skin surface temperature, which may influence the barrier-related parameters [29, 32].

The hydration level of skin surface can be accurately determined using Corneometer® CM 825 (Courage & Khazaka) by measuring electrical capacity as the alternating voltage of SC. Figure 2 shows the measurement principle of Corneometer® CM 825. The higher the water content in epidermis, the higher is its electrical capacity [33], resulting in higher value of SC. Adequate skin hydration is vital to maintain a healthy skin, which makes moisturiser an important component in basic skin care. Table 2 below represents the interpretation of skin hydration.

3.3. Skin melanin and erythema

Skin colour is predominantly determined by pigments such as hemoglobin, bilirubin carotene and mostly, melanin. Melanin is the main characteristic to differentiate ethnic types. Skin pigmentation primarily evolves to regulate UV radiation, penetrating skin by controlling its biochemical effects and can be significantly altered by substances, such as drugs and irritants [35]. Figure 3 illustrates the measurement principle of Mexameter® MX18.

Erythema is highly associated to skin redness. It occurs along with skin injury, inflammation, or infection. In general, erythema can be caused by infection, acne medication, exercise, massage, allergies, solar radiation (sunburn), cutaneous radiation syndrome (acute radiation exposure to skin) leading the capillaries in the skin to dilate (hyperemia), resulting in skin redness [37].

Mexameter® MX18 is a device to measure the quantities of two major components responsible for skin colour, namely melanin and hemoglobin (erythema). The measurement is based on the absorption and reflection

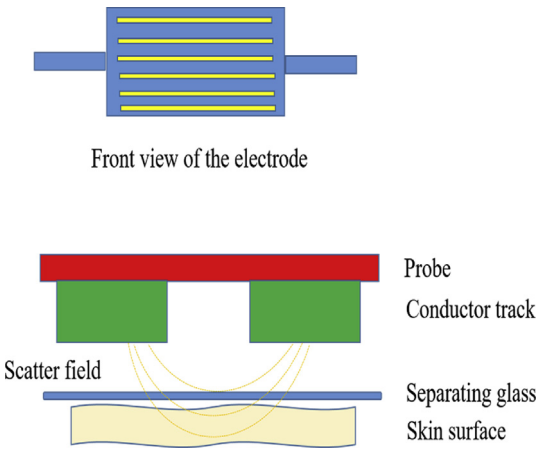

Figure 2. Illustration of the measurement principle, Corneometer® CM 825 (Modified figure from [34]).

of an active colour detecting chip. Briefly, melanin is measured using two wavelengths that are chosen according to different absorption peaks of melanin pigments, while erythema measures are used to estimate the redness level (hemoglobin) in skin.

3.4. Skin elasticity

The mechanical properties of skin can be assessed by evaluating the thickness and qualitative properties of epidermis, dermis, and subcutis. Aging causes qualitative and quantitative changes in skin, such as loss of elasticity, reduction in the epidermal thickness and collagen content, increased production of wrinkles as well as pigment lesions. However, these features may vary among individuals [38]. Figure 4 illustrates the measurement principle of Cutometer®.

Skin elasticity is measured by suction with respective probes according to Nilsson's Vapour Pressure Gradient Method with Cutometer® (Courage & Khazaka) probes. The Cutometer® is designed to measure the elasticity of the upper skin layer using negative pressure which mechanically deforms the skin. Suction is generated to produce negative pressure which consequently draws the skin into the aperture of the probe. The penetration depth inside the probe is evaluated by a non-contact optical measuring system. Figure 5 shows the skin elasticity changes which are categorised based on age.

3.5. Skin collagen

Collagen is a type of protein manifested in the skin, bone, tendon, cartilage, and blood vessels. They are predominantly rich in glycine, proline, and hydroxyproline [40]. Human dermis consists primarily 70% collagen to provide good support, maintain elasticity and tensile as well as to reinforce skin structure in order to appear smooth and young.

Skin high resolution ultrasound by DermaLab® Combo (Cortex Technology) has been widely employed for skin scanning. Ultrasonic imaging relies on the properties of reflected sound waves through the tissue. Different tissues reflect waves distinctively due to the variations in tissue structure, vascularity, and density, which are highly correlated to the differences of collagen, keratin, and water content [41]. Translating this fact to dermatology, dermis that appears echogenic (transducers of 20 MHz) with echoes originating from the fibre network can be considered to comprise elastic fibres, collagen, and tissue atrophy. Figure 6 shows the illustration of the measurement principle using ultrasound skin imaging.

3.6. Skin sebum

Sebum lipids on the skin surface have major impact on the protective and mechanical properties of the epidermal barrier. Sebumetry (Sebumeter® SM 815; Courage & Khazaka) is generally used to quantify the sebum production on the skin surface. In brief, a special tape will become transparent after physical contact with the sebum on the skin surface. The translucency of the tape will be measured using a photometry system. The light permeability of the tape changes after 30 s of skin contact, depending on the sebum content on the skin surface [42]. Figure 7 depicts the measurement principle of Sebumeter® SM 815. Table 3 represents the result of skin sebum interpretation.

3.7. Skin pH

The pH value of skin is associated with the quality of hydrolipid film. Basically, the pH of stratum corneum regulates three epidermal functions, which are antimicrobial barrier, permeability barrier homeostasis, and barrier integrity/cohesion. The pH alterations on stratum corneum could lead to abnormal epidermal barrier function [44]. Skin pH Meter® PH 905 (Courage & Khazaka) is a probe consisting of a flat-topped glass electrode (to enhance skin contact) connected to a voltmeter which is specifically designed to determine the pH values of skin. In general, the

Table 2. Interpretation of skin hydration results [23].

Moisture value	Body Parts	
	Forehead, cheek, chin	Hand, arms
Very dry	<30	<5
Dry	30–60	5–25
Sufficiently moisturized	>60	>25

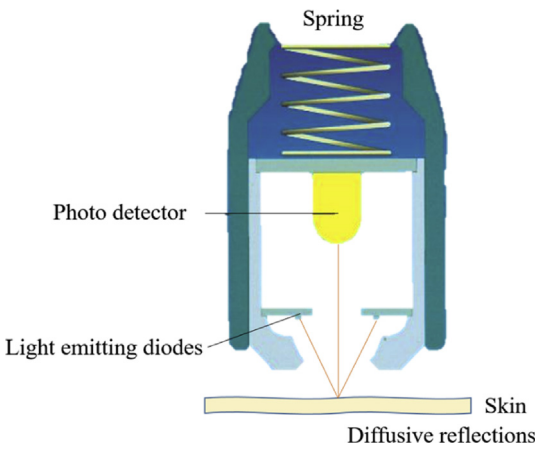

Figure 3. Illustration of the measurement principle, Mexameter® MX18 (Modified figure from [36]).

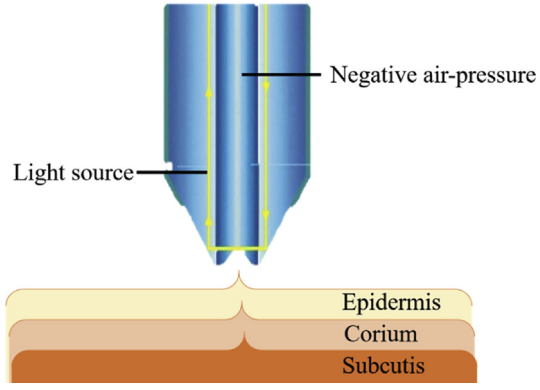

Figure 4. Illustration of the measurement principle, Cutometer (Modified figure from [39]).

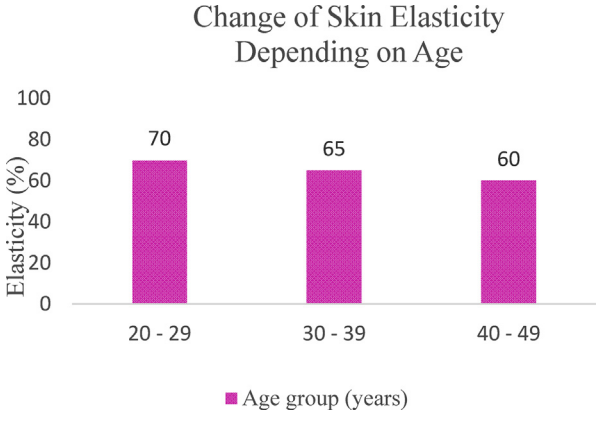

Figure 5. Change of skin elasticity depending on age groups [31].

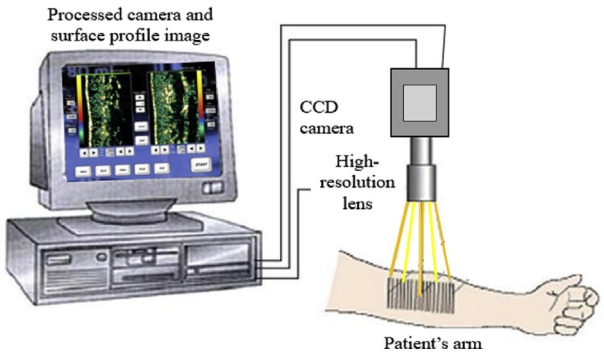

Figure 6. Illustration of the measurement principle using ultrasound skin imaging (Modified figure from [41]).

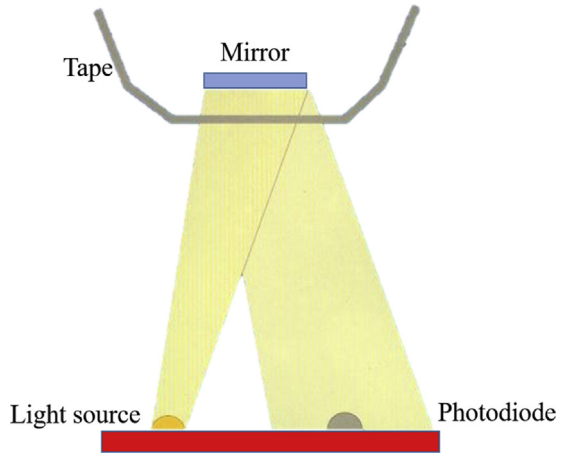

Figure 7. Illustration of the measurement principle, Sebumeter® SM 815 (Modified figure from [43]).

Table 3. Interpretation of skin sebum results [23].

Moisture value	Body Parts	
	Forehead, cheek, chin	Hand, arms
Very dry	<30	<5
Dry	30–60	5–25
Sufficiently moisturized	>60	>25

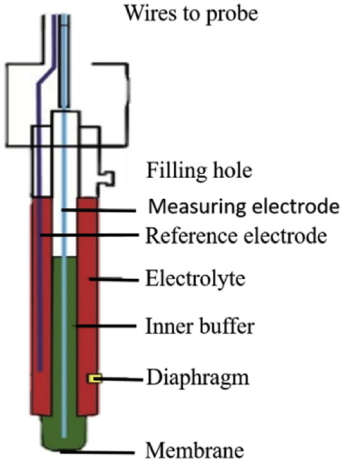

Figure 8. Illustration of the measurement principle, Skin pH Meter® PH 905 (Modified figure from [46]).

Table 4. Interpretation of skin pH results [23].

pH	<3.5	3.8	4.0	4.3	4.5	5.0	5.3	5.5	5.7	5.9	6.2	6.5	>6.5
Women	+ Acidic range -				Normal				- Alkaline range +				
Men	+ Acidic range -				Normal				- Alkaline range +				

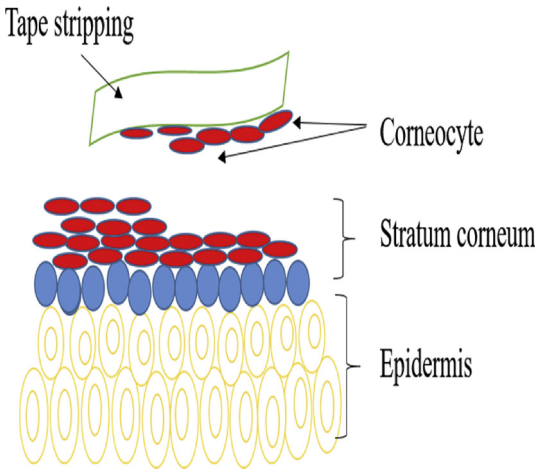

Figure 9. Schematic of the tape stripping test (Modified figure from [70]).

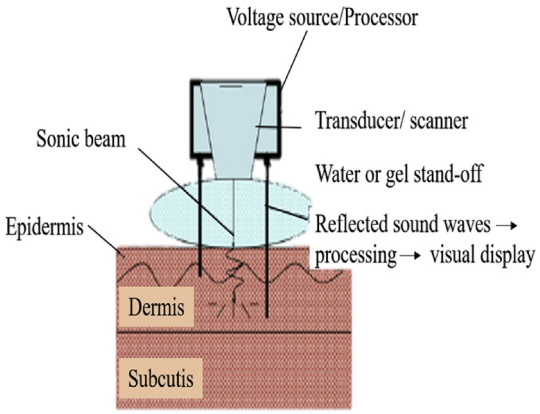

Figure 10. Depiction of ultrasound device, including voltage source, transducer, water or gel standoff, and sonic beam projected into skin (Modified figure from [81]).

activity of hydrogen cations is measured based on its adjacency to the thin layer of hydrated gel which is located on top of the probe [45]. Figure 8 shows the measurement principle of Skin pH Meter® PH 905. Table 4 represents the interpretation of skin pH results.

4. The non-invasive assessment of skin intercellular lipid profiling

4.1. Tape stripping

Tape stripping that involves the subsequent removal of SC using adhesive tapes has emerged as a useful technique to study the physiology of SC in recent years [47, 48]. The stripping tape is placed onto the subject's skin and slowly removed. Flakes that are stuck on the tape surface can be observed under optical microscope. This method is simple, non-invasive, requires no chemical consumption, and allows the collection of different layers of corneocytes separately (via sequential application of the tape to the same area of the skin) [49].

Tape stripping is a universal method and can be applied in *in vivo* [50, 51, 52] and *in vitro* studies [53, 54, 55]. The amount of SC removed by a

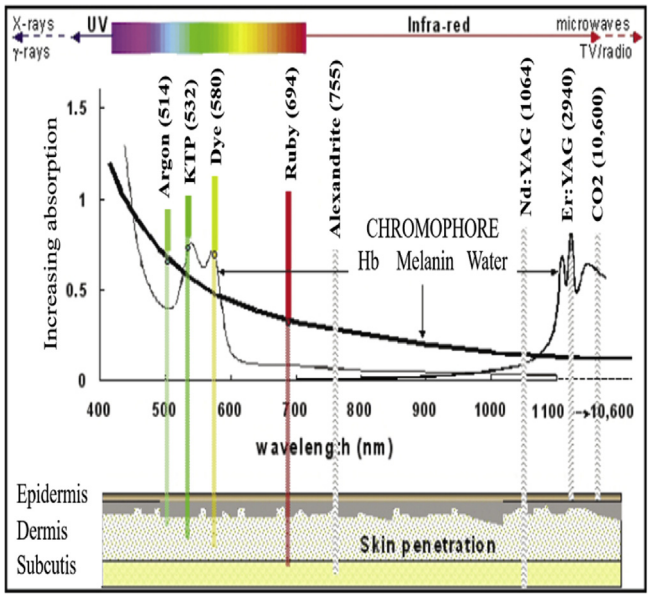

Figure 11. Comparative absorption spectra showing common laser wavelengths and their depth of skin penetration (Modified figure from [89]).

single adhesive tape strip depends on several intrinsic factors, such as the number of cell layers [56] and corneocytes [57], the thickness of SC [58] as well as the composition and amount of lipids [59]. The amount of SC removed varies on the anatomical site. Tape stripping method is a relatively fast and simple technique, which is suitable for large-scale studies in humans [60].

Previous studies conducted on the different layers of SC demonstrate a significant increase of phospholipids and marked decrease in total ceramides as compared with more superficial ones [61]. In contrast, Weerheim and Ponec [62] found no ceramide gradient using the same method in healthy volunteers. However, it can be concluded that the ratio of cholesterol/ceramide in the inner and outer SC has no significant difference [63]. Denda *et al.* studied the influence of tape stripping on the quantitative amount of lipids in SC reported an increase in ceramides 1 and 2 and a decrease in other types of ceramides, which clarify the way in which these substances affect scaly skin [64].

The amount of protein is directly correlated to the amount of SC removed using tape stripping [65, 66]. The amount of SC removed by each strip literally decreased as the SC is progressively stripped [67, 68, 69]. The adhesion properties of the adhesive and the cohesiveness of the corneocytes strips will determine the removed amount of SC mass. Approximately, one-third of strips amount is needed to remove 45–50% of SC [60]. Figure 9 shows the schematic of the tape-stripping test.

4.2. Ultrasound

In recent years, the applications of ultrasound in dermatology have attracted a lot of attention due to the development of machines that work with high- and multiple-frequency of probes to allow the optimal definition of superficial structures [71]. Ultrasound is one of the non-invasive methods in skin imaging technologies which can be applied to objectify the shape and size of any structure. Ultrasound is a simple and reproducible technique to measure the skin thickness [72], inflammatory conditions, tissue edema, and the extent of dermal and subcutaneous

Table 5. Lists of herbal based product used non-invasive techniques.

No.	Type of product	Specification herbal materials	Solvent of Extraction	Herbal Used in Product		Species/Model	Number of subjects	Mean age	References
1.	Moisturizer	Commercial products	N/A	HM1	Jojoba, vit E	Normal humans	40	40 ± 9 y/o	[111]
				HM2	Chamomilla recutita, helanthus annuus, sambucus nigra, primula veris, theobroma cacao				
				HM3	Hydolyzed elastin, talc, tocopheryl acetate				
				HM4	Aloe barbadensis				
				HM5	Elaeis guineensis, olea europaea, persa fratissima, prunus armeniaca, ribes nigrum, vitis vinifera, micro fruit oil				
				HM6	Shea butter, cocos nucifera, olea europaea fruit oil (olive), aloe barbadensis (leaf)				
				HM7	Vit E, vit A, theobroma cacao, pollen extract, triticum vulgare (wheat germ oil)				
				HM8	Cucumis sativus juice, coumarin, hexyl cinnamal, limonene				
				HM9	Aloe vera, indian madder, country mallow				
				HM10	Kapoor kachari, chandan, nimba, ghrit kumari, ushir, gulabjal, tulasi, haridra, yastimadhu, malai, grape seed oil, olive oil, badam oil, keshar, bhavpralash, tankan amla (boric acid), rastarangni				
				HM11	Santalum album (sandal wood), cuscus grass (vetiveria zizanioides), sweet basil (ocimum sanctum), aloe vera, honey				
				HM12	Behda kwath, madhu, ankurit gehum, kusumbhi tail, methi beej, vach				
				HM13	Olive oil, sesame oil, Vit E				
				HM14	Olive oil, red apple				
				HM15	Aloe vera, jojoba oil, milk cream, wheat germ				
				HM16	Vit A, D, E, Aloe vera, wheat germ oil, rose water				
				HM17	Almond, sandal wood, honey, wheat germ oil, jojoba oil, essential oil of patchouli, germanium, rose and basil				
				HM18	Grape seed, wheat germ oil, vit E, vit F				
				HM19	Cocoa butter, vit E, aloe vera extract				
				HM20	Honey, almond				

(continued on next page)

Table 5 (*continued*)

No.	Type of product	Specification herbal materials	Solvent of Extraction	Herbal Used in Product	Species/Model	Number of subjects	Mean age	References
2.	Moisturizer	Different concentrations (0.135–0.9% w/w) of extracts, juice and gel	Ethanol:water	Aloe barbadensis (Leaf) Glycerriza glabra (Bark) Cucumis sativus (Fruit) Trigonella Foenum Graecum(Seed) Triticum sativum(oil) Cocos Nucifera(oil) Prunus Amygdalus(oil) Oleum olivae(oil) Azadirachta indica(Leaf) Santalum Alba(oil) Emblica officinale	Humans with history of dry and itchy skin	20	30 ± 10 y/o	[112]
3.	Topical formulation	(6% w/w) Glycolic *Ginkgo biloba* extract or glycolic green tea extract	Ethanol	Ginkgo biloba, green tea	Albino hairless mice (male)	24	Not indicate	[113]
4.	Cream	3% of the concentrated extract of Basil	Ethanol	Basil	Normal humans (male)	11	48 y/o	[114]
5.	Powder (orally administered)	4.0 g Atractylodes lancea rhizome, 4.0 g Hoelen, 3.0 g Cnidium rhizome, 3.0 g Japanese Angelica root, 2.0 g Bupleurum root, 1.5 g Glycyrrhiza root, and 3.0 g Uncaria thorn.	Purified water	Yokukansan (*Atractylodes lancea* rhizome, Hoelen, *Cnidium* rhizome, Japanese Angelica root, *Bupleurum* root, *Glycyrrhiza* root, and *Uncaria* thorn)	Mice (male)	-	10 weeks	[115]
6.	Cream	5% concentrated extract of *T. chebula*	N/A	Terminalia chebula	Normal humans (male)	11	30 y/o	[116]
7.	Topical applications	20 μL of 0.1% (about 0.67 mg/kg body weight) apigenin	Ethanol	Chrysanthemum	Hairless mice (female)	-	6–8 weeks old	[117]
8.	Topical formulation	60 µl of 2% hesperidin	Ethanol	Orange (Peel)	Hairless mice Fed mouse diet (female)	-	6–8 weeks old	[118]
9.	Essential oil	3% w/w essential oil of *R. alba*	N/A	Rose	Wistar rats (Male) Normal humans (women)	- 14	11 weeks old 21.0 ± 0.1 y/o	[119]
10.	Lotion	0.1%, 0.05% and 0.01% (v/v of 1% Eucalyptus extract)	Ethanol:water Methanol:water	Eucalyptus	Normal humans (Female & male)	18	33 y/o	[120]
11.	Essence & serum	N/A	N/A	Prinsepia utilis and purslane	Acne vulgaris patients	83	Not indicate	[121]
12.	Powder (orally administered)	N/A	N/A	Radix rehmanniae, radix scrophulariae, radix ophiopogonis, poria, rhizoma dioscoreae, fructus corni, rhizoma alismatis, radix paeoniae alba, cortex moutan	Dermatitis patients	100	Not indicate	[122]
13.	Topical formulation	6.0% w/w of Camellia sinensis glycolic leaf extract	N/A	Camellia sinensis (leaf)	Normal humans (Female)	24	25–40 y/o	[123]
14.	Topical formulation	0.1% apigenin	N/A	Chrysanthemum	Hairless mice (Female)	-	6–8 weeks old	[124]
15.	Cream	Different concentrations (0–15.00 % w/w)	water/propylene glycol	Seaweed thalli	Normal humans (Female)	10	27 y/o	[125]
16.	Cream	3% of *M. oleifera* leaf extract	N/A	Moringa (leaves)	Normal humans (male)	11	20-35 y/o	[126]
17.	Cream	N/A	Organic solvent	Terminalia arjuna	Postmenopausal patients (Female)	60	50–70 y/o	[127]
18.	Cream	Different concentrations (4–5 % w/w)	N/A	Pomegranate seed oil, grape seed oil, sesame oil, flower honey	Normal humans (Female)	12	25-65 y/o	[128]
19.	Cream	0.5% *C. indicum* extract	Methanol	Chrysanthemum indicum (flowers)	Normal humans (Female)	30	41–50 y/o	[129]
20.	Powder (orally administered)	N/A	Ethanol:water	Panax ginseng Meyer	Normal humans (Female)	98	40-60 y/o	[130]
21.	Cream	N/A	N/A	Panax ginseng and Crataegus pinnatifida	Normal humans (Female)	21	30-65 y/o	[131]
22.	Topical formulation	N/A	Ethanol	Panax ginseng Meyer	Hairless mice	32	6 weeks old	[132]

fibrosis as well as to monitor the course of wound healing [73, 74, 75]. Only ultrasound with 50MHz high resolution transducer can determine the image of the epidermis [76]. The ideal morphology of the skin should require the absence of contact between the device and the skin, the images recorded at video rate, a depth of the skin thickness, spatial resolution, and a volume vision [77].

Moreover, high variable-frequency ultrasound is an advanced technique that produces quantitative and qualitative information on the skin lesions and surrounding tissues. It is capable to define deeper structures of the skin layers and perfusion patterns in real time. A previous study by Wortsman and Wortsman reported that skin ultrasound is a highly effective adjuvant to diagnose skin lesions by clearly separating the lesional from the extralesional areas, exogenous from the endogenous components, and dermatologic from the nondermatologic conditions. Therefore, it is safe to say that non-invasive ultrasound imaging provides highly relevant clinical information that can be a fundamental technique to study human skin [78].

Ultrasound was first applied in dermatology as a fixed-frequency equipment (20–100 MHz) that was able to distinguish the layers of skin; hence, several studies on cutaneous pathologies have been performed using this method [79]. Ultrasound is able to provide reasonable balance between penetration and resolution, real-time capability as well as the possibility to identify and measure both texture and blood flow changes [80]. However, despite the remarkable properties of ultrasound, it can only measure 0.1 mm lesions and detect pigments such as melanin epidermal lesions [78]. Figure 10 depicts the ultrasound device, including voltage source, transducer, water or gel standoff, and sonic beam projected into the skin.

4.3. Laser confocal microscopy

Confocal microscopy or known as confocal laser scanning microscopy (CLSM) was inaugurated in 1991 by New *et al.* [82]. It is a high-resolution optical detection technique [83] that provides impressive confocal images of cellular organisation in human skin [84]. The device basically visualises living epidermal cells individually and primarily focuses on natural contrast, hydration state, and environment. Besides, it is also able to measure the SC thickness at micrometer level and repair cutaneous wounds.

A number of specific interactions could occur when the laser light comes into contact with the skin surface. The light source focuses on a small volume of sample, which makes it hard and unsuitable to access a large field of view. To overcome this drawback, the confocal technique removes all focused backscattered photons from the surrounding. Laser beam that is not transmitted will be absorbed by the tissue or any material and generate heat energy which can cause thermal damage to the tissue [85]. The depth of transmission into the tissue depends on the tissue type, laser wavelength, and laser fluency [86].

However, to reduce the damage from laser exposures, several preventive steps have to be considered and taken into account. For example, appropriate safety goggles must be worn to filter the specific wavelengths of laser light during an operation [87]. Cloth drapes should be wet with sterilised water or saline solution, while metal instruments are usually burnished or ebonised to decrease laser light reflection. Other than that, protective cylinders and shields should be attached to the end of the hand piece to absorb fumes, vaporised particles, and splattered blood and tissue [88]. Hazard on flammability around the treatment site is of great concern. Figure 11 shows the comparative absorption spectra along with the common laser wavelengths and their skin penetration depth.

In contrast, reflectance-mode confocal microscopy (RMCM) is a non-invasive technology with the same principle as CLSM. However, RMCM role is limited to superficial epidermal wounds as well as angiogenesis [90] and to identify skin morphology condition (normal or abnormal) [91]. To say the least, confocal microscopy potential in vivo study is very promising as compared to other modern technologies [92, 93].

5. The recent case study used non-invasive techniques in dermatology

The world has witnessed the application of non-invasive techniques in dermatology mainly due to their tremendous benefits and great ability to determine the skin lipid structure. However, the standardisation of non-invasive methods is a concern to get a successful output. Therefore, various factors should be taken into account to obtain reproducible and relevant results. This paper provides information that can be optimised, and thus be a great benefit to apply non-invasive techniques in dermatological studies. In contrast, to evaluate the skin functions, full range results can be embraced by employing several non-invasive techniques (Table 5). Many previous research had reported the potential mechanisms by which herbal plants can improve the skin physiological and intercellular lipid properties. The utilisation of herbal plants could be a valuable alternative approach to prevent and/or treat skin disorders. Table 5 summarised the application of non-invasive techniques in herbal plant studies.

6. Conclusion

This paper reviewed several non-invasive techniques to examine the skin physiological conditions, epidermal lipid profiles, and evaluation of herbal-based product efficiency. The advantages and limitations of each of the methods are briefly discussed. Although the marketplace is flooded with a diverse array of commercial skin analysis tools, it is important to use the most appropriate techniques to measure skin properties depending on the skin issues. The development of advanced non-invasive diagnostic techniques allows tissue imaging *in vivo* and contributes to a more accurate diagnosis of skin diseases.

In recent years, there has been an increasing interest on non-invasive techniques in clinical and investigational dermatology. The classical methods have substantially improved, leading to the development of novel tools and provide a growing number of biophysical methods to assess skin properties. The availability of non-invasive techniques in dermatology shows substantial differences concerning their limitations and opportunities, potential clinical applicability and practicability. Future research should aim to improve the technical limitations and investigate the impact of combining two or more techniques in order to enhance the diagnostic impact.

Declarations

Author contribution statement

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