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Short Communication

An *In-vitro* evaluation of skin protection factor of non-polar date seed extract from three different date varieties Ajwa, Aseel and Khapra

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Abstract

Objective: Skin is the primary layer of human body which act as protective barrier against exogenous chemicals, UV radiations as well as microorganisms. Maintaining the skin morphology under regular environmental stresses and controlling age related skin changes is one of the biggest challenges for modern science, pharmaceutical and cosmetics industry. These environmental stresses, such as excessive exposure to UV radiations, can even lead to the devastating skin disorders such as skin cancer. Dates seed has capability to protect skin against the damages caused by solar radiations, which mostly appear in the forms of wrinkles and some other skin related issues. Methods: Therefore, current research is focused on to analyze sun protection factor (SPF) of two non-polar fractions obtained from crushed seeds of three different varieties of i.e., Ajwa, Aseel and Khapra. Results: Results indicate that the highest value of SPF was found in AEA i.e., 15.061 at 200ppm while no difference was observed in the SPF values of KPPE. Conclusion: Hence, it could be concluded that the ethyl acetate fraction of all date seed varieties could be potential Ajwa, Aseel and Khapra could be a promising source of cosmetic and pharmaceutical preparations due to the potential SPF value except petroleum ether of Khapra extract.

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Introduction: Skin has an enormous biological significance as well as cosmetic effect i.e., acting as a first line of defense against foreign agents to provide vibrant external look to the person. Although, skin aging is a normal biological phenomenon which remain acceptable unless or until it leaves visible marks on the skin which sometimes impact negatively on ones' psychology. Currently, early aging is one of the most discussed topics which often related to the direct and indirect environmental stresses including solar radiations. Approximately, 1,000,000 people per annum are prone to skin cancer and most of them died due to malignant melanoma. Skin cancer commonly occur in exposed body parts such as head, face, neck and hands [1-4].Covering human body is not only for a beautification, but since ancient times, it is also being used as a protective shield on the skin against sun exposure[5].

Predominantly, the solar radiations, which composed of ultraviolet (UV) region of the electromagnetic spectrum, impart deleterious effects on the skin. Ultraviolet (UV) region contains three categories i.e., UVA (400-320 nm); UVB (320-290 nm) and UVC (290-200 nm). UVA radiation penetrates into the deep layers of the epidermis and dermis which, in turn, evokes the generation of free radicals, a primary factor responsible for premature skin ageing. UVB radiations, can also cause sunburn and form pyrimidine dimmers which leads to the skin damage. Approximately, 65% of the skin damages are caused by UVB radiations. UVC radiations, on the other hand, unable to reach earth and blocked completely by **Ozone layer**. UV radiation expose cause acute and chronic skin conditions effects such as sunburns, photo-aging and cancers. To counter such detrimental effects of sun-exposure, researchers has formulated various body creams and lotions, with sunscreens as an active ingredient, to protect skin from deleterious effects of UV-radiations[6,7].

In the late 19th century, the first scientific report was published on the use of photo-protective agents. In 1974, the term SPF became popular worldwide, which was introduced by Greiter and previously known as "Schulze method". Earlier, every sunscreen product contains variable amount of active ingredient due to unavailability of standardization method to calculate SPF values. This had led lack in synergy among individual products. In 1978, Food and Drug Regulatory Authority (FDA), proposed the first ever SPF standardization method. As per the FDA defined method, SPF (which is used to express potency of sunscreen) can be calculated as follows[8,9].

$$SPF = \frac{\text{UV energy required to produce a minimal erythema dose (MED) on protected skin}}{\text{the UV energy required to produce a MED on unprotected skin.}}$$

The minimal erythemal dose (MED) is defined as threshold dose that may produce erythema/sunburn[10]. The higher the value of SPF, the more efficacious will be the product against sunburn. However, it should be kept into consideration that standardization of technique/method, used for identification or quantification of SPF of products, is necessary [1,11,12]. With the development of *in-vitro* screening methods for sunscreens, it has become easier to evaluate the possible photo-protection strength of sunscreen molecules or mixtures. In general, two types of methods are commonly being utilized in order to evaluate quality of sunscreen products. Firstly, measurement of UV absorption or transmission pattern of sunscreen product films via quartz plates/bio-membranes. Secondly, spectrophotometric analysis of diluted sunscreen solution[13,14].

In context with the effectiveness of different sunscreens, some myths and misconception needed to be taken into consideration. For instance, it is generally accepted that SPF-60 is twice as effective as SPF-30 in preventing sunburns but, in actual, it is not. It is only mentioned due to certain laboratory conditions. It should be noted that, SPF-30 sunscreen absorbs 96.7% of UV rays, while SPF-60 sunscreen absorbs 98.3%. That is mean, SPF-60 is only 1.6% more effective than SPF-30[5,15]. Recently, naturally occurring compounds and formulations have been widely accepted as a potential source of sunscreen because of their absorption in the UV region [16-18]. It has been reported that date palm seeds had shown significant anti-ageing properties in women with substantial decrease in the skin wrinkles[19,20], while the fat content within date seed helps to prevent contact dermatitis [21]. Furthermore, palm seed oil (Degletnour and Allig seed oil) can also protect cellular damage against UV-B and UV-A radiation. Therefore, sunburn formulation utilizing date seed oil as UV protector can also be formulated[22,23] .

Methodology:

Plant Material: Ajwa date fruits (fully ripe) were purchased from the market of the Holy city of Madinah-tul-Munnawwarah, Saudi Arabia. Khapra date fruits were purchased from the orchid of Khairpur, Pakistan. These dates were dispatched to Karachi with proper packaging and storage conditions. Aseel date fruits (fully ripe) were purchased from the market of the Karachi, Pakistan. The specimen has submitted at Centre for Plant Conservation, Karachi University, Herbarium and Botanic Garden, Karachi, Pakistan for identification bearing a voucher# 92173. The date specie and its plant parts were identified by Ms. Afsheen Athar of Department of Botany, University of Karachi. Karachi dates will also be subjected to identification as per the approved protocols[24].

Extraction: Seeds were separated from the pulp and then sun-dried for 2 days. After that, seeds were crushed and converted into granular form and weighed properly. For experimentation, 100gm of crushed seeds of Ajwa, Aseel and Khapra had been subjected to sequential extraction with petroleum ether and ethyl acetate, to lead fractions i.e., APE, AEA, KAPE, KAEA, KPPE and KPEA respectively.

Skin Protection Factor (SPF) analysis: The measurement of protective effect of sunscreen from ultraviolet-B rays was assessed by skin protection factor. Briefly, three different dilutions of extract sample (200ppm, 100ppm and 50ppm) in chloroform were prepared and absorbance was taken at 290nm, 295nm, 300nm, 305nm, 310nm, 315nm and 320nm. Chloroform was used as a blank. All fractions were run in triplicate.

Result and discussion: Sunscreens are widely used cosmetics now days for various reasons either for skin protection from light, aging, photo carcinogenesis or management of many skin diseases. Broad spectrum protection against ultraviolet rays is the primary goal of the consumers. Herbal cosmeceuticals are another emerging trend of the new world that drawn the attention of researchers to explore the use of medicinal plants in this

field. Aim of the current study is to explore three different varieties of date for their skin protection properties. Dates seeds extract were screened for Skin Protection Factor (SPF) analysis. The ethyl acetate extract of Ajwa date seed (AEA) has the highest SPF value at 200ppm i.e., 15. 061.Whereas, ethyl acetate of Khapra date seed (KPEA) and petroleum ether of Ajwa date seed (APE)at 200ppm, possess almost similar SPF values i.e.,14.315 and 14.006 respectively. As per the EC recommendation, AEA extract lie in the medium category and has SPF-15. Petroleum ether and ethyl acetate extract of Aseel date seed (KAPE and KAEA) at 200ppm has SPF of 10.528 and 10.343 respectively. Similarly, ethyl acetate extract of Ajwa date seed (AEA) also evaluated at lower concentration (100ppm) and showed SPF values of 10.488. It means that AEA extract is more potent and effective than KAPE and KAEA against sunburns. The petroleum ether extract of Khapra date seed (KPPE) showed the lowest SPF value (2.725) at 200ppm with non-significant variation at different concentrations as represented in the above table.

The result shows that AEA, has the most promising potential to be used in sunscreen product than APE, KAEA, KAPE and KPEA. In addition, all varieties of date seed extract (expect KPPE) are qualified and could be used in cosmetics and pharmaceutical applications.

Conclusion: There are several herbal products that showed promising a photo-protective effects. Date seeds extract showed a good skin protection against UV exposure. They prevent sunburn by extensive UV exposure topically. They have good safety profiles, although these have generally not been as well-studied. More research is needed to explore photo-protective nature of this medicinal plant.

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Table. 1. Recommendations of the European Commission (EC) highlighting four protection classes moderately than the SPF numbers[5].

S.No.	Labelled category	Skin protective factor (SPF)	Ultraviolet A radiation- protection factor (UVA-PF)
1.	Low protection	6 10	The degree of UVA protection is related to the SPF value. The UVA-PF must be greater than 1/3 of the SPF
2.	Medium protection	15 20 25	
3.	High protection	30 50	
4.	Very high protection	+50	

Table. 2. SPF activity of different seed extracts of *Phoenix dactylifera* L.

S.No.	EXTRACT	CONC.	SPF
1	KAPE	200 µg /ml	10.528±3.28
		100 µg /ml	4.356±2.89
		50 µg /ml	1.692±1.07
2	APE	200 µg /ml	14.006±1.38
		100 µg /ml	8.79±8.65
		50 µg /ml	2.703±7.22
3	KPPE	200 µg /ml	2.725±0.92
		100 µg /ml	2.238±0.51
		50 µg /ml	1.809±0.24
4	KAEA	200 µg /ml	10.343±7.38
		100 µg /ml	5.653±4.43
		50 µg /ml	2.472±2.01
5	AEA	200 µg /ml	15.061±2.50
		100 µg /ml	10.488±1.40
		50 µg /ml	3.939±2.46
6	KPEA	200 µg /ml	14.315±1.84
		100 µg /ml	7.811±7.93
		50 µg /ml	4.634±1.28