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Assessment of Photoprotective, Antioxidant and Anti-Skin Cancer Activities of Leaf Extracts of Certain Medicinal Plants

Amal A.A. Mohamed#, Wafaa A.A. Sorour
Botany Department, Faculty of Science, Aswan University, Aswan, 81528, Egypt.

PLANT extracts acquiring antioxidant potential have recently been used for topical applications for significant protection against ultra violet (UV)-induced sunburn. The current study was conducted for screening *Pluchea discoridis* (Asteracea), *Lawsonia inermis* (Lythraceae), *Aloe vera* (Liliaceae) and *Eucalyptus camaldulensis* (Myrtaceae) for their photoprotective, antioxidant and anti-skin cancer properties.

Multivariate data analysis based on spectrophotometric evaluation of major secondary metabolites was performed. Each plant had its specific pattern of metabolites. *P. discoridis* and *E. camaldulensis* were separated from other plants due to their high content of secondary metabolites where *P. discoridis* was discriminated by high content of flavonoids and saponins. *E. camaldulensis*, was discriminated by high content of phenolics and tannins.

Different concentrations of plant extracts were read for absorbance at UVB spectrum. Sun protection factors (SPFs) were calculated. The highest concentration (10mg/mL) of *P. discoridis* extract had the highest SPF, *i.e.*, 23.94± 0.98.

The antioxidant activity using 2,2-Diphenyl-1-picryl-hydrazyl (DPPH) assay revealed that *P. discoridis* and *L. inermis* extracts had the best free radical scavenging activity with DPPH at 100µg/mL of 85.69± 1.8 and 85.35± 2.5%, respectively.

Human skin tumor cell line (A431) was incubated with the plant extracts. *P. discoridis* had the best anti-skin cancer activity of lowest LC_{50} value. The effect induced by the *P. discoridis* extract was also identical to that of the standard chemotherapeutic drug (doxorubicin).

The present study revealed that *P. discoridis* is a promising plant for use in sunscreen formulations and anti-skin cancer treatment.

Keywords: Anti-skin cancer, Antioxidants, Cosmetics, *Pluchea discoridis*, Sun protection factor (SPF), Sunscreens.

Introduction

The living organisms are regularly subjected to an array of various exogenous threats, e.g., the exposure to ultraviolet (UV) solar radiation. The electromagnetic spectrum in the UV region can be divided into three wavelength band-regions, UV-A (400-320nm), UV-B (320-290nm) and UV-C (< 290nm). UV-C radiation is blocked by the atmosphere, while, UV-B radiation is not totally penetrated out by the ozone layer. The increased levels of UV-B radiation getting to the surface of the Earth due to ozone depletion adversely affects human health (Singh & Sharma, 2016).

The skin provides a major anatomical barrier in humans against the damaging effects of solar radiation. About 65% of the skin damage is caused by UV-B radiation (Mbanga et al., 2014).

Sunscreens are chemical products that have the ability to protect from the undesirable

#Corresponding author email: amal.mohamed2@aswu.edu.eg
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effects of solar and in particular UV radiation (Elmets & Anderson, 1996). The efficacy of a sunscreen depends on its sun protection factor (SPF) which is simply could be expressed by the following formula:

SPF= Minimal erythema dose in sunscreen (MED) on protected skin)/ Minimal erythema dose on unprotected skin .....(1)

Also, MED in Equation (1) could be defined as the lowest time interval required to produce a minimal apparent erythema on unprotected skin (Wood & Murphy, 2000).

The greater the SPF, the more efficient is the product in avoiding the skin from sunburn. The spectrophotometry is used for *in vitro* determination of SPF of any product.

Plants are major sources for natural products which are considered as potential cosmetics, *i.e.*, sunscreens as a result of their absorption for UV radiation and their antioxidant activity (Khazaeli & Mehrabani, 2010).

L. inermis, *A. vera* and *E. camaldulensis* are inextricably used for cosmetic and medicinal purposes in Asia and North Africa (Semwal et al., 2014). Literature scarcity about *P. dioscoridis* has motivated the investigation of this species in the present study.

Pluchea dioscoridis L. belongs to Asteraceae. It is the most common Pluchea species reported in Egypt. The plant was found to have potent antimicrobial activity (Zain et al., 2012).

Lawsonia inermis L. is also known as Henna belongs to the Lythraceae. Henna is traditionally used to protect the hands and hair against fungal pathogens (Gull et al., 2013).

Eucalyptus camaldulensis belongs to the Myrtaceae. It is an indigenous species to Australia and Tasmania and well-known for its medicinal uses due to the rich content of oil in their leaves (Sani et al., 2014).

Aloe vera belongs to Liliaceae and it has been used in folk medicine. *A. vera* is a succulent plant adapted to accommodate in areas suffering from water scarcity. Species of *Aloe* are of potential biological properties as therapeutic botanicals (Patel et al., 2012).

In the prevailing study, extracts of *P. discoridis*, *L. inermis*, *A. vera* and *E. camaldulensis* were selected for their UV absorption and SPF values. In addition, their antioxidant and skin anticancer activities were assessed.

Materials and Methods

Chemicals and equipment

All chemicals were of analytical quality. 2,2-Diphenyl-1-picryl-hydrazyl (DPPH) was purchased from Sigma Chemical Co. All biological assays were done under sterilized conditions using a cabinet biosafety class II level (Baker, SG403INT, Sanford, ME, USA).

Spectrophotometric measurements were acquired using Shimadzu UV-Visible Spectrophotometer, Model UVmini-1240 (Shimadzu Corporation, Japan) and microplate multi-well reader (Bio-Rad Laboratories Inc., model 3350, Hercules, California, USA).

Collection and preparation of plant materials

Pluchea discoridis and *E. camaldulensis* were collected from the arid zone around the campus of Aswan University (23°59'56"N, 32°51'36"E). They were taxonomically authenticated according to Täckholm (1974) and Boulos (2009). Leaves of *P. discoridis* and *E. camaldulensis* were separated from the plants and air-dried. *A. vera* plants were cut from cultivated plants from the botanical garden of Aswan University. The succulent leaves of *A. vera* were peeled and the gel was collected in a petri dish and left to be air-dried. The dried powdered leaves of *L. inermis* were purchased from the local market. The voucher numbers of the herbarium specimens of *L. inermis*, *P. discoridis* and *E. camaldulensis*, to which the collected samples were complemented, are (007181, 008864 and 011643 ASW/HER/BOT, respectively).

Extraction of plant materials

The dried powdered plant materials were extracted three times with 90% methanol. The total extracts were filtrated and the solvent was dried under vacuum (40ºC) and the obtained solid mass (the crude extract) was used for subsequent determination of major bioactive

secondary metabolites and biological activity assays.

Determination of major bioactive secondary metabolites

Determination of flavonoids

The content of total flavonoids was determined according to Zhishen et al. (1999). $NaNO_2$ (5%) solution was added to the plant extract and 6 min later, it was mixed with Al $(Cl)_3$ (10%). The mixture was allowed to stand for 6 min at ambient temperature and 1M NaOH was added. The absorbance was read at 510nm. The content of flavonoids was calculated as mg quercetin equivalent/ g extract.

Determination of total phenolics

The content of total phenolics was determined based on Folin-Ciocalteau method (Singleton et al., 1999). One mL of Folin-Ciocalteau reagent was mixed with 1mL of each extract. Then, 1mL of Na_2CO_3 (10%) was mixed and vortexed. The absorbance was read at 700nm after 1hr. Gallic acid was used as a standard and treated as samples. The content of total phenolics was calculated from the standard curve as mg Gallic acid equivalent/ g extract.

Determination of saponins

According to Ebrahimzadeh (1998), 5mL of vanillin reagent (2%) in H_2SO_4 were added to 1mL of each extract or with purified saponin standard and all were incubated at 60ºC. After 1hr, the samples and standards were put in an ice bath for 10min. The absorbance was read at 473nm, and the saponin content was calculated as mg saponins equivalent/ g extract.

Determination of tannins

The content of total tannins was determined using vanillin assay (Julkunen-Tiitto, 1985). Vanillin (4% in methanol) was added to plant extract. Concentrated HCl was added to the mixture and left to stand for 20min at ambient temperature. A series of catechol standard was treated as above. The absorbance was read at 550nm, and the content of total tannins was expressed as mg catechols equivalent/ g extract

Screening of photoprotective activity

Photoprotective activity was evaluated by analyzing the plant extract for the *in vitro* SPF.

The crude extracts were dissolved in DMSO. Scanning spectra of different concentrations of plant extracts were attained by running from 320 to 290nm. Mathematical equation created by Mansur et al. (1986) was applied for the calculations (Equation 2).

$$SPF = CF \times \sum_{290}^{320} EE(\lambda) \times I(\lambda) \times Abs(\lambda) \quad \text{.....(2)}$$

where: *EE* is the erythemal effect spectrum; *I* is the solar intensity spectrum; *Abs* is the absorbance of sunscreen product; *CF* is a correction factor (= 10). The values of *EE* × *I* are constants measured by Sayre et al. (1979), and are shown in Table 1.

TABLE 1. Constants used in the calculation of SPF (Sayre et al., 1979)

Wavelength (λ nm)	EE×I (normalized)
320	0.0180
315	0.0837
310	0.1864
305	0.3278
300	0.2874
295	0.0817

Screening of antioxidant activity

The *in vitro* antioxidant activity was measured by the radical scavenging method using the DPPH assay. Stock solutions of plant crude extracts (1mg/ mL in DMSO) were prepared.

Two mL of DPPH solution (0.1mM) were added to 1mL of different concentrations of the plant extracts (in DMSO). The absorbance was measured at 517 nm after incubation for 30min in the dark (Manzocco et al., 1998). The percentage of the DPPH radical scavenging was determined using Equation (3) as given below:

$$\%\ inhibition\ of\ DPPH\ radical = \left[\frac{Abs\ control - Abs\ sample}{Abs\ control}\right] \times 100 \quad \text{..(3)}$$

where, Abs control is the absorbance of DPPH radical + DMSO; Abs sample is the absorbance of DPPH radical + plant extract.

The calculated IC_{50} value represents the concentration that is required for 50 % inhibition of DPPH.

Screening of anti-skin cancer activity

The effect of the tested medicinal plants on cell viability was measured according to the method described by Mosmann (1983). Human skin tumor cell line (A431) was incubated with different concentrations of the plant extracts (0.78, 1.56, 3.125, 6.25, 12.5, 25, 50 and 100µg/ mL in DMSO) for 48hrs. The colorimetric MTT assay was used for assessing the number of viable cells according to Thabrew et al. (1997). A concentration of 100µg/mL of a known cytotoxic natural agent was used as positive control, which causes 100% lethality under the same conditions. The absorbance was measured using a microplate multi-well reader at 595nm and a reference wavelength of 620nm.

The percentage of alteration in viability was determined using the formula depicted in Equation (4).

$$\left(\left(\frac{Absorbance\ of\ plant\ extract}{Absorbance\ of\ negative\ control}\right)-1\right)\text{x}\,100 \quad ..(4)$$

Statistical analysis

Results were expressed as Mean ± SD. Data of spectrophotometer analysis were subjected to multivariate data analysis such as principal component analysis (PCA) and hierarchical clustering analysis (HCA) using Minitab (version 18.1, LEADTOOLS ©1991-2002, LEAD Technologies, Inc). The possible two-way linear relationships among determined metabolites and photoprotective, antioxidant and anti-skin cancer activities were assessed using Pearson's correlation test. One-way ANOVA, with Tukey correction was applied to determine significant differences for the comparison. Probit analysis was used for LC_{50} and LC_{90} determination using SPSS 11 program.

Results

Metabolic profile for major bioactive secondary metabolites

The tested plants were dominated with high content of flavonoids, phenolics, saponins and tannins. The contents of these measured secondary metabolites were significantly different in the plant extracts (Fig. 1).

The content of total flavonoids was significantly high in *P. discoridis* L., *inermis* and *E. camaldulensis* compared to *A. vera* (F-value= 17.56; P< 0.001) (Fig. 1).

The content of total phenolics was significantly high in the plant extract (F-value= 22.00; P< 0.001). The highest content of total phenolics was measured in the leaf extract of *E. camaldulensis* (176.4± 14.54mg gallic equivalent/ g extract), while, the lowest value was (79.62± 23mg gallic equivalent/ g extract) measured in the leaf extract of *L. inermis* (Fig. 1).

The content of total saponins was significantly high in *P. discoridis* compared to *L.*, *inermis* and *E. camaldulensis A. vera* (F-value= 48.24; P< 0.001). The content of total saponins in *P. discoridis* was equal to 245.05± 19.36mg saponin equivalent/ g extract (Fig. 1).

The content of total taninns was significantly different in the plant extracts (F-value= 11.07; P< 0.01) (Fig. 1).

Data obtained from spectrophotometer were subjected to the PCA and HCA to show the similarities or dissimilarities between the four plant extracts (Fig. 2). Each one from the tested plants showed particular pattern of bioactive secondary metabolites (Fig. 2 A). *P. dioscroidis* and *E. camaldulensis* were separated from other group by having very high amounts of all detected secondary metabolites where they are placed in the right hand side of the ellipse (positive part of PC1). In comparison between *P. dioscroidis* and *E. camaldulensis*, *P. dioscroidis* was characterized with higher content of saponins than *E. camaldulensis* which was characterized with higher content of tannins and phenolics. *L. innermis* and *A. vera* were placed in the left hand side of the ellipse (negative part of PC1) and both plants were characterized with lower amounts of all detected secondary metabolites than the other group (*P. dioscroidis* and *E. camaldulensis*). So the dendrogram (hierarchical clustering analysis) showed a close relation between *L. innermis* and *A. vera* (Fig. 2 B).

Photoprotective activity

The absorption profile of the plant extracts was depicted in Table 2. The different concentrations

of the extracts of *P. discoridis* and *L. inermis* showed the highest values of absorbance at the UV-B spectrum (320- 290nm). The ultraviolet absorption spectrum for most plant extracts showed two peaks of maximum absorption (300-305nm) (Table 2).

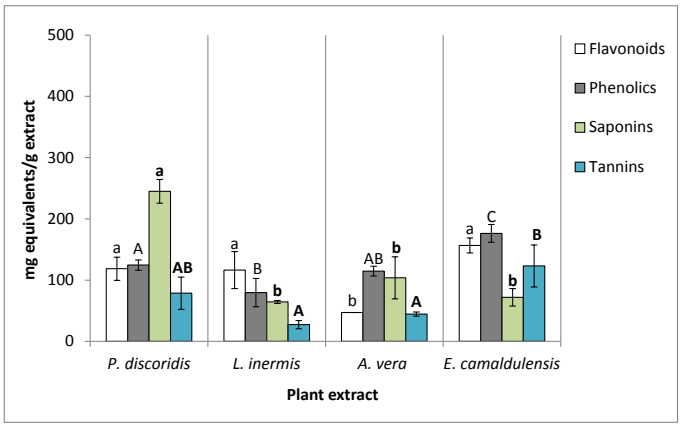


Fig.1. Phytochemical analysis of *Pluchea discoridis, Lawsonia inermis, Aloe vera* and *Eucalyptus camaldulensis*.

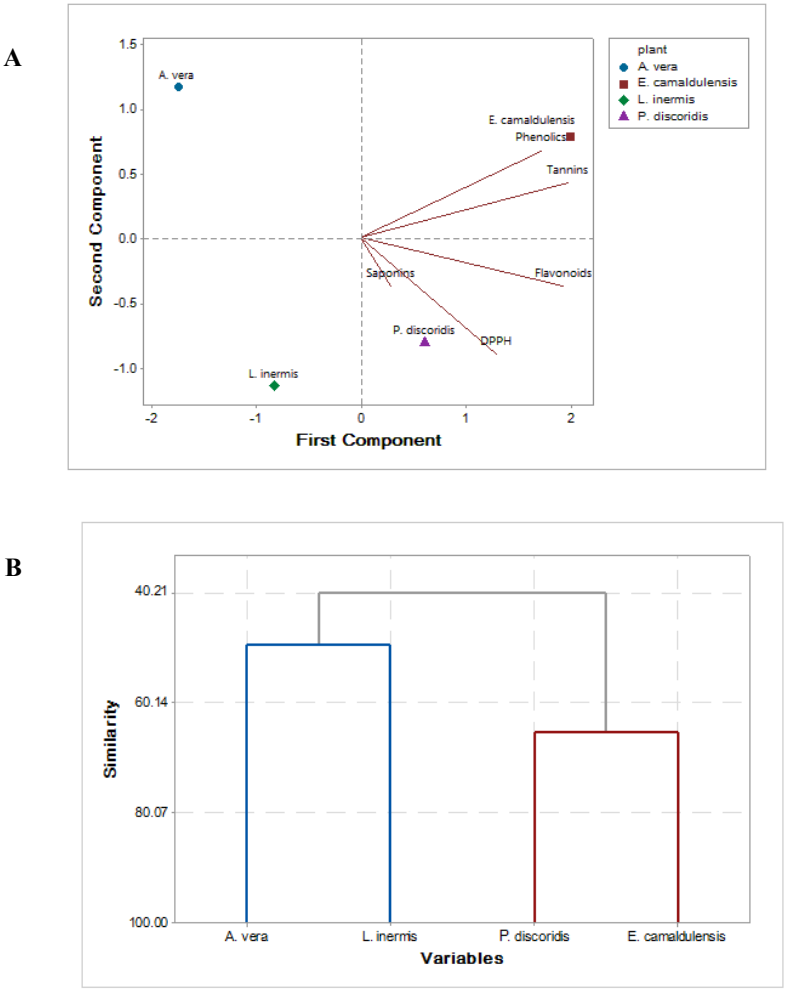


Fig. 2. Principal Component Analysis (PCA) of the metabolic data obtained from spectrophotometer of *Plucea discoridis, Lawsonia inermis, Aloe vera* and *Eucalyptus camaldulensis.* Score biplot (A) and hierarchical clustering analysis (B)

TABLE 2. UV-absorbance and calculated values of sun protection factor (SPF) for different concentrations of the plant extracts.

Wavelength (λ nm)	*P. discoridis*	*L. inermis*	*A. vera*	*E. camaldulensis*	TiO_2
1mg/ mL					
320	1.46±0.06	1.58±0.01	1.38±0.01	0.99±0.07	0.12±0.01
315	1.65±0.15	1.76±0.12	1.56±0.06	0.99±0.01	0.13±0.01
310	1.69±0.12	1.76±0.01	1.63±0.01	1.10±0.08	0.16±0.02
305	1.82±0.05	2.05±0.01	1.92±0.02	1.99±0.05	0.29±0.01
300	2.15±0.05	2.20±0.06	2.12±0.05	2.25±0.01	0.81±0.01
295	1.99±0.01	2.04±0.03	1.98±0.00	2.04±0.01	1.66±0.01
290	1.59±0.02	1.65±0.03	1.62±0.00	1.69±0.02	1.52±0.02
SPF	**18.79±2.01**	**19.97±0.78**	**18.85±1.10**	**17.97±1.24**	**5.31±0.21**
2mg/ mL					
320	1.24±0.01	1.67±0.12	1.53±0.07	1.72±0.05	0.09±0.01
315	1.38±0.01	1.87±0.01	1.71±0.13	1.93±0.27	0.18±0.01
310	1.49±0.01	1.87±0.13	1.74±0.11	1.89±0.18	0.25±0.01
305	2.35±0.15	2.08±0.01	1.86±0.05	1.93±0.05	0.30±0.01
300	2.47±0.01	2.20±0.06	2.13±0.04	2.17±0.05	0.92±0.08
295	2.05±0.02	2.08±0.01	1.99±0.02	2.02±0.01	1.62±0.03
290	1.69±0.02	1.52±0.10	1.59±0.00	1.60±0.01	1.54±0.01
SPF	**20.88±1.03**	**20.41±0.98**	**19.05±1.03**	**19.91±1.09**	**5.81±0.31**
5mg/ mL					
320	2.05±0.07	1.33±0.01	1.26±0.01	1.63±0.02	0.12±0.04
315	2.30±0.24	1.45±0.00	1.36±0.00	1.79±0.07	0.25±0.01
310	2.23±0.01	1.56±0.01	1.44±0.03	1.82±0.01	0.43±0.03
305	2.50±0.01	2.37±0.08	2.37±0.12	2.06±0.01	0.51±0.01
290	1.62±0.02	1.67±0.01	1.68±0.01	1.59±0.02	1.83±0.06
300	2.41±0.03	2.39±0.01	2.45±0.02	2.22±0.06	1.40±0.30
295	2.07±0.03	2.02±0.01	2.04±0.01	2.02±0.01	1.9±0.13
SPF	**23.49±1.05**	**20.89±0.67**	**20.78±1.05**	**20.19±2.08**	**8.56±0.82**
10mg/ mL					
320	2.19±0.29	2.43±0.02	0.69±0.02	1.80±0.09	0.18±0.01
315	2.45±0.01	2.45±0.03	0.68±0.00	1.97±0.00	0.29±0.04
310	2.35±0.21	2.43±0.07	0.71±0.02	1.99±0.09	0.51±0.08
305	2.52±0.04	2.35±0.13	0.70±0.00	2.17±0.01	0.61±0.01
300	2.40±0.03	2.36±0.09	0.73±0.02	2.26±0.02	1.73±0.04
295	2.10±0.02	2.07±0.01	0.73±0.02	2.09±0.02	2.89±0.03
290	1.54±0.13	1.62±0.02	0.67±0.11	1.53±0.08	2.61±0.01
SPF	**23.94±0.98**	**23.42±2.11**	**7.12±1.09**	**21.21±0.88**	**10.9±0.01**

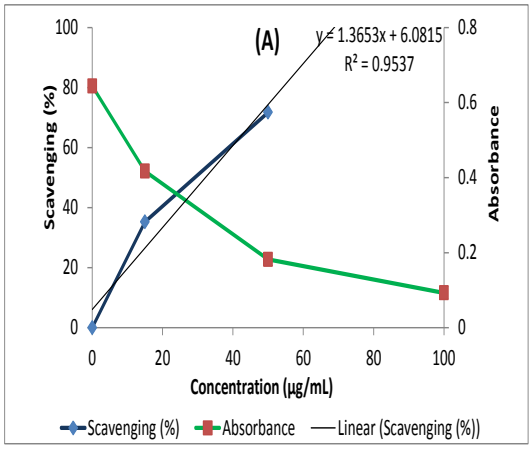
The values of SPF of the different concentrations of the extracts of the tested plants were calculated according to Equation (2). The results of the absorption profile and SPF of the plant extracts were compared to those of TiO_2. For most plant extracts and TiO_2, values of the absorbance and SPF were concentration-dependent. The values of SPF for TiO_2 ranged from 5.31± 0.21 to 10.9± 0.01 for concentrations 1 to 10mg/mL, respectively.

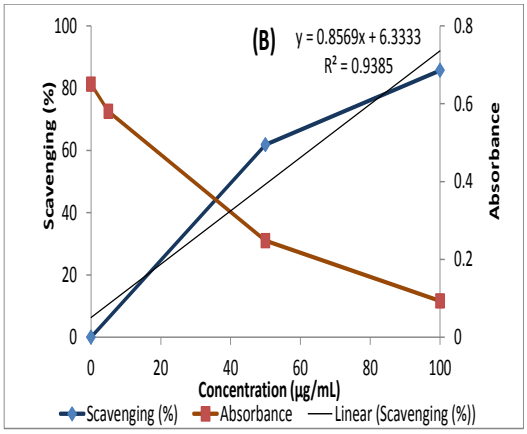
Only in *A. vera*, the highest concentration of the extract (10 mg/mL) showed the lowest value of SPF (7.12± 1.09) when compared to the different concentrations of the plant extracts. The SPF values of the other plants ranged from 17.97±1.24 to 23.94±0.98 (Table 2).

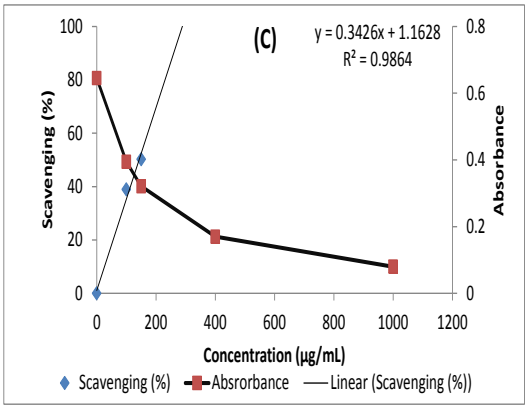
Antioxidant activity using DPPH assay

The antioxidant activity of the plant extracts was measured by radical scavenging assay using the DPPH method as previously described. The method depends on the reduction of DPPH in presence of an antioxidant and the gradual change of DPPH colour from violet to yellow depending on the concentration of the antioxidant which is indicated by reduction in the absorbance.

The results revealed that the scavenging of DPPH was correlated to the concentration of the extracts of the plants. The relation between the concentration and antioxidant activity (absorbance and scavenging of DPPH) of the plants extracts was shown in Fig. 3. The extract of *A. vera* was the lowest in antioxidant activity compared to other plants and increasing of the extract concentration was required to construct the standard curve (Fig. 3 C).







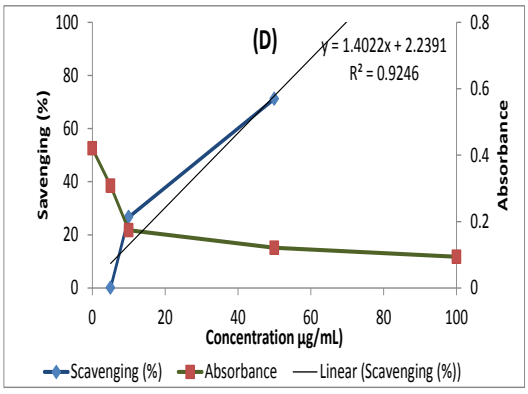


Fig. 3. The *in vitro* antioxidant activity of the plant extracts: (A) *Plucea discoridis*, (B) *Lawsonia inermis*, (c) *Aloe vera*, (d) *Eucalyptus camaldulensis* and using DPPH assay.

The values of DPPH (at 100µg/mL of plant extract) were measured using DPPH assay and the values of IC_{50} were calculated using the reprehensive regression line equation and both were used to compare the antioxidant potency of the plants (Table 3). The lower the value of IC_{50}, the higher the activity of the antioxidant. The data showed that *P. discoridis* extract exhibited the

best free radical scavenging activity with a value of IC_{50} of 32.16µg/mL, followed by the extract of *E. camaldulensis* exhibiting a value of 34.06µg/ mL for IC_{50} (Table 3). The antioxidant activity of the extracts was compared with ascorbic acid as the standard antioxidant. Ascorbic acid had a value of IC_{50} equal to 7.50µg/ mL (Table 3).

Effect of the plant extracts on A431 skin cancer cells lines

Results of MTT assays showed that the plant extracts decreased the percent viability of the cells. The LC_{50} and LC_{90} values of the plant extracts against A431 skin cancer cell lines are represented in (Table 4). The lowest values of LC_{50} and LC_{90} indicate the highest anti-cancer activity of the plant extract.

Regarding these values, extract of *P. discoridis* was found to stimulate more cytotoxicity towards skin cancer cell line A431 when compared to the other tested plant extracts (Table 4). It possessed the lowest values LC_{50} and LC_{90} of 25.2 and 46.3µg/ mL, respectively. The effect induced by the *P. discoridis* extract was also comparable to that of the standard chemotherapeutic drug (doxorubicin, the positive control) where it exhibited 100% lethality when applied at 100µg/ mL (Table 4). The extract of *E. camaldulensis* followed that of *P. discoridis* in the anti-skin cancer activity of values equal to 52.3 and 88.4 for LC_{50} and LC_{90}, respectively.

Correlation between determined secondary metabolites and biological activities

Pearson's correlation was performed to summarize two-way linear relationships among different variables and photoprotective, antioxidant and anti-skin cancer activities (Table 5).

It was indicated that SPF was highly significantly correlated at $P< 0.001$ (negative correlation) to both phenolics and tannins ($r= -0.918$ and -0.817, respectively).

A strong significant positive correlation ($P< 0.001$) was found between DPPH and flavonoids. Also, flavonoids had a negative significant correlation with IC_{50} ($r= -0.869$) which indicated that higher antioxidant activity was related to higher flavonoids content (Table 5).

Higher anti-skin cancer activity was related to higher content of tannins and saponins indicated by a strong significant negative correlation between LC_{50} and LC_{90} in one side and tannins and saponins in the other side (Table 5).

TABLE 3. The values of IC_{50} and DPPH (%) of the plant extracts (µg/ mL) using DPPH assay.

Plant	IC_{50} (µg/mL)	DPPH % (at 100 µg/mL)
P. discoridis	32.16	85.69 ± 1.8^{a}
L. inermis	50.95	85.35 ± 2.5^{a}
A. vera	142.54	38 ± 0.8^{c}
E. camaldulensis	34.06	77.61 ± 3.2^{d}
(Ascorbic acid)	7.50	----------

Letters on bars indicate significant difference ($P < 0.05$).

TABLE 4. The *in vitro* anti-skin cancer activity of the plant extracts against A431 cell line using MTT assay.

Plant	LC_{50}[1] (µg/ mL)	LC_{90}[2] (µg/ mL)	Lethality % at 100 µg/ mL
P. discoridis	25.2	46.3	100%
L. inermis	87.8	138.9	56.4%
A. vera	62.9	109	75.3%
E. camaldulensis	52.3	88.4	91.3%
Doxorubicin (Positive control)	28.3	48.8	100%
DMSO	--------	--------	3%
Negative control	--------	--------	0 %

[1]LC_{50}: Lethal concentration of the sample that leads to the death of 50% of cells in 48hrs
[2]LC_{90}: Lethal concentration of the sample that leads to the death of 90% of cells in 48hrs

TABLE 5. Correlation coefficients (r) between determined metabolites and bioactivity in the plant extracts

	Flavonoids	Phenolics	Saponins	Tannins	SPF	DPPH	IC_{50}	LC_{50}
Phenolics	0.55							
	*							
Saponins	-0.009	0.054						
	ns	ns						
Tannins	0.648	0.896	0.166					
	**	***	ns					
SPF	-0.285	-0.918	-0.13	-0.817				
	ns	***	ns	***				
DPPH	0.779	0.009	0.212	0.221	0.165			
	***	ns	ns	ns	ns			
IC_{50}	-0.869	-0.261	-0.227	-0.446	0.104	-0.963		
	***	ns	ns	ns	ns	***		
$L_{C5}0$	-0.175	-0.512	-0.826	-0.543	0.634	-0.142	0.298	
	ns	*	***	*	**	ns	ns	
LC_{90}	-0.232	-0.496	-0.837	-0.546	0.603	-0.223	0.372	0.997
	ns	*	***	**	**	ns	ns	***

P value 0.001 0.003 0.02 0.064 0.067 0.068 0.089 0.1 0.146 0.234 0.347 0.413 0.467 0.477 0.49 0.49 0.509 0.585 0.605 0.66 0.868 0.978

ns= Non-significant; * and ** = Significant at P< 0.1 and P< 0.05, respectively; ***= Highly significant at P< 0.001.

Discussion

Ultraviolet radiation induces oxidative stress and inflammatory responses which harmfully affect the skin along with premature photoaging that ultimately leads to skin cancer (Khazaeli & Mehrabani, 2008; Vilela et al., 2011; Patil et al., 2015; Gajardo et al., 2016; Napagoda et al., 2016) with the realization of their adverse side effects, the recent trend is to search for human friendly alternative formulations especially of plant origin. Therefore, the present study focuses on evaluation of photoprotective activity of aqueous extracts (1mg/mL. The use of antioxidants is a protective strategy to reduce the injurious effects of sun exposure. The antioxidants from natural origin are beneficial photoprotectives against the harmful effects of UV radiation. Recently, the protective effect of plant extracts against the photoaging and photocarcinogenesis was studied (Polonini et al., 2014; Cefali et al., 2016; Szychowski et al., 2018).

In the present study, the phytochemical screening of *P. discoridis*, *L. inermis*, *A. vera* and *E. camaldulensis* revealed the presence of a high content of bioactive secondary metabolites, i.e, phenolics, flavonoids, saponins and tannins. These metabolites were known to exhibit biological activities (Brewer, 2011; Patel et al., 2012; Sani et al., 2014; Ashour et al., 2019).

Plants and their derived compounds act as the prospective photoprotective resources due to their ability of UV absorbing in the UV-B region (Korać & Khambholja, 2011; Takshak & Agrawal, 2019). In the present study, *P. discoridis*, *L. inermis* and *E. camaldulensis* showed high ability of UV absorbance in a concentration dependent manner. Moreover, the UV absorption spectrum showed peaks of maximum absorption (300-395nm). These results were in accordance to those obtained by Dutra et al. (2004) who indicated the maximum absorbance of flavonoids at peaks (240-280nm; 300-500nm). In the same context, extracts of other medicinal plants have verified their effectiveness of being wide-ranging spectrum sunscreens indicating a high UV absorbance (Polonini et al., 2014; Singh & Sharma, 2016; Mota et al., 2020).

The SPF is a quantitative measurement of the efficacy of a sunscreen formulation and its value is

labeled on the commercially available lotion and cream products (Hupel et al., 2011; Skocaj et al., 2011; Gajardo et al., 2016). Spectrophotometry measurements are used to find the actual SPF of these products. The labeled SPF values of most sunscreen products were found to be higher than the actual SPF (Mbanga et al., 2014).

In the current study, the calculated SPFs of the plant extracts of *C. discoridis*, *L. inermis* and *E. camaldulensis* were higher than the SPF of TiO_2. Higher SPFs calculated for different herbal extracts in previous studies were similar to those of the present study (Malsawmtluangi et al., 2013; Costa et al., 2015; Singh & Sharma, 2016).

Ingredients with antioxidant properties in the sunscreens give a broad protective spectrum. Antioxidants of natural origin are promising candidates for enhancing sun protection of sunscreens. Hence, the addition of plant-derived antioxidants to sunscreens is a rising interest to provide dual protection against oxidative stress and UV (Bambal et al., 2011; Polonini et al., 2014; Mann et al., 2020).

The present investigation showed that the measurement of antioxidant activity regarding the values of IC_{50} of the extracts indicated a high potency of antioxidant activity of *C. discoridis*, *L. inermis* and *E. camaldulensis*. Previous studies indicated the importance of antioxidant activity in UV protection. Furthermore, high SPF values were found to be correlated to high flavonoid and phenolic contents of plant extracts (Brewer, 2011; Ebrahimzadeh et al., 2014; Silveira et al., 2020).

For many centuries, plants have been a rich source for therapeutic purposes. However, plant extracts were investigated for antitumor activity from only few decades (Steenkamp & Gouws, 2006; Marchetti et al., 2012; Nayak et al., 2015).

Skin cancer is one of the major causes of mortality worldwide (Balakrishnan & Narayanaswamy, 2011; Valachovic & Zurbenko, 2014). Development of novel strategies for treatment is required due to the increasing of skin distortions (Thuncharoen et al., 2013).

Plant extracts have been assayed for anti-skin cancer activity (Korać & Khambholja, 2011). In the present experiment, the antitumor activities against skin cancer of crude extracts of *C. discoridis*, *L. inermis*, *A. vera* and *E. camaldulensis* were recorded for the first time. The results indicated that *P. discoridis* had the highest antitumor activity against the human skin tumor cell line (A431). The obtained results are supported by previous studies where plant extracts rich in flavonoids had a strong inhibition of the growth of A431 cell lines (Mohansrinivasan et al., 2015; Neerugatti et al., 2016; Zeng et al., 2018). Also, the anti-skin cancer activity of *P. discoridis* was higher than that of the standard chemotherapeutic drug (doxorubicin). The results indicated that *P. discoridis* could be a putative source of natural substitute for chemotherapeutic drugs.

Conclusion

This investigation revealed that *P. discoridis* is a promising candidate for use in sunscreen formulations due to its high content of bioactive secondary metabolites which contribute to its integrative sun protective, antioxidant and anti-skin cancer activity when compared with the selected medicinal plants which are traditionally used for skin and hair care.

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Authors contribution: AAA Mohamed: Conceptualization, formal analysis for spectrophotometer, statistical analysis and writing the first draft of the manuscript. WAA Sorour: Providing facilities, editing and reviewing the manuscript. All authors have read and agreed to the submitted version of the manuscript.

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تقييم الحماية الضوئية من أشعة الشمس ومضادات الأكسدة وأنشطة مكافحة سرطان الجلد في مستخلصات أوراق بعض النباتات الطبية

أمل على عوض الله محمد، وفاء عبد الوهاب عابد سرور
قسم النبات- كلية العلوم- جامعة أسوان - ص ب ٨١٥٢٨ أسوان - مصر

تم مؤخرًا استخدام المستخلصات النباتية في التطبيقات الموضعية للحماية من الحروق الناجمة عن الأشعة فوق البنفسجية. ولذا أجريت الدراسة الحالية لفحص الخصائص الواقية ومضادة الأكسدة ومضادة السرطان لنباتات البرنوف *Plucea discoridis* والحناء *Lawsonia inermis* والصبار *Aloe vera* والكافور *Eucalyptus camaldulensis* .

تم إستخدام تحليل البيانات متعدد المتغيرات بعد إجراء التحليل الطيفى للمركبات الأيضية الثانوية. تم تمييز *P. discoridis* و *E. camaldulensis* عن النباتات الأخرى، حيث تم تمييز *P. discoridis* من خلال المحتوى العالي من الفلافونيدات والصابونين، وكذلك تم تمييز *E. camaldulensis* من خلال المحتوى العالي من الفينولات والتانينات.

تم قراءة الإمتصاص في طيف UVB للمستخلصات النباتية.وكذلك تم حساب معاملات الحماية من الشمس (SPFs), ووجد أن تركيز (10 مجم / مل) من *P. discoridis* كان له أعلى معامل بمقدار 0.98 ± 23.94.

كشف إختبار نشاط مضادة الأكسدة ((DPPH عند تركيز (010 مجم / مل)، أن . *P. discoridis* و *L. inermis* لهما أفضل كسح للشوارد الحرة بمقدار 85.69 ± 1.8 و 2.5 ± 85.35٪ على التوالي.

تم تحضين الخلايا السرطانية لورم الجلد (A431) مع المستخلصات النباتية. وجد أن *P. discoridis* أفضل مضاد سرطان الجلد بأقل قيمة لـ LC50. كذلك التأثير الناجم عن *P. discoridis* متطابقًا أيضًا مع العلاج الكيميائي القياسي (دوكسوروبيسين).

كشفت الدراسة أن *P. discoridis* هو نبات واعد لاستخدامه في مستحضرات واقية من الشمس ولعلاج سرطان الجلد.