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NOTE

In vitro evaluation of UV opacity potential of *Aloe vera* L. gel from different germplasms

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Abstract In this study, lyophilized crude and methanolic extracts of aloe gel from different germplasms (S24, RM, TN, OR, and RJN) of *Aloe vera* L. were tested for their ultraviolet (UV) opacity potential. UV absorption profiles, sun protection factor (SPF), and percentage blocking of UVA and UVB were considered to test UV opacity potential. Both the extracts showed UV absorption and followed the same path in the wavelength range of 250–400 nm in all the germplasms. Methanolic extract showed a stronger absorptivity than the crude lyophilized extract. Among the tested germplasms, maximum UV opacity property with a SPF of 9.97% and 79.12% UVB blocking was obtained with RJN, whereas a poor response was evident in TN with a SPF of 1.37% and 28.5% UVB blocking at 4 mg/ml methanolic extract. To our knowledge the present work for the first time documents UV opacity properties of *A. vera* L. gel and opens up new vistas in *Aloe* gel characterization.

Keywords *Aloe vera* L. · UV absorption · Sun protection factor · UV blocking

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Introduction

Exposure of human skins to ultraviolet (UV) radiation is increasing because of depletion of the stratospheric ozone layer. Chronic exposure to UV may cause sunburn, skin cancer, oxidative stress as well as photoaging [1–4]. Numerous epidemiological investigations show that protection from solar UV radiation will reduce the risk of acute and chronic skin damage in humans. There has been increasing interest in protection against solar UV radiation using various types of sunscreen. UV opacity is the property of sunscreens which absorb and/or block UV radiations from reaching the skin. The biological activity of a sunscreen is evaluated by its ability to protect human skin from erythema and is represented by a sun protection factor (SPF) [5, 6]. The SPF provides an index of protection against erythemally effective solar UV, largely confined to the UVB (290–320 nm) and short-wavelength UVA (320–340 nm) region [7]. In order to avoid expensive and time-consuming in vivo UV protection testing, in vitro method for SPF measurement using spectral transmission has been developed. [5]. The spectral transmittance of a sunscreen in the ultraviolet spectral range can be used to predict an in vitro SPF value based on standard erythema and solar data [5, 6].

Aloe vera L. (syn. *A. barbadensis* Miller) belonging to the family Liliaceae has been known traditionally as the "healing plant" and used in a variety of cosmetic product formulations. The mucilaginous gel of this plant has been shown to have wound healing [8], anti-inflammation [9], immunostimulatory [10, 11], and antioxidant properties [12]. However, in spite of its use as an ingredient in myriad health and cosmetic products the UV opacity potential of aloe gel has not yet been demonstrated. Plant extracts have recently been considered as a potential source of

sunscreens due to their absorption in UV regions as well as antioxidant properties [13, 14]. Root extract of *Pothomorphe umbellata* and extracts from lichens and boldo tree were found to have antioxidant and photostability properties [15, 16]. The protection factors as well as the good UV light absorption of their photoproducts suggest the use of natural substances in sunscreen preparations.

The goals of the present work are to evaluate the UV absorption potential and to determine the in vitro sun protection factor of aloe gel obtained from different germplasms of *A. vera* L.

Materials and methods

Plant materials

Germplasms of *A. vera* L. (*A. barbadensis* Miller) were collected from different agroclimatic zones of India. The plant material was identified by Prof. G. G. Maity, Taxonomist, University of Kalyani and was grown in the Agricultural and Food Engineering Departmental farm of IIT Kharagpur, India. The five studied germplasms are designated S24, RM, TN, OR, and RJN.

Extract preparation

Healthy and fresh leaves having a length of 30–45 cm were collected from 10- to 12-month-old plants and were washed with distilled water to remove dirt. After removing the spikes, the leaves were cut transversely into pieces and the thick epidermis was carefully separated from the parenchyma using a scalpel-shaped knife. The resulting mucilaginous gel was homogenized and freeze-dried with a yield of 2.5, 2.1, 0.9, 1.8, and 1.5 g, respectively, for the germplasms S24, RM, TN, OR and RJN. To obtain methanolic extracts, 1 g of lyophilized gel was mechanically agitated in 80 ml 80% methanol for 4 h. The mixture was then centrifuged at 10,000 rpm for 15 min. The supernatant was evaporated under reduced pressure at 40°C to obtain the sticky brown methanolic extracts (300, 232, 104, 240, and 206 mg, respectively, for the germplasms S24, RM, TN, OR, and RJN). Test samples were obtained by dissolving lyophilized crude and methanolic extracts in phosphate buffer saline (PBS, pH 7.4).

UV opacity analysis and determination of in vitro sun protection factor

The spectral properties of aloe gel were tested initially with the germplasm S24 at 1, 2, and 4 mg/ml concentrations and were measured using UV–VIS–NIR spectrophotometer (Perkin-Elmer, USA, Model Lamda-900) for the wavelength range 280–400 nm (UV-A, 320–400 nm; UV-B, 280–320 nm). Among the tested concentrations, 4 mg/ml was found to be more opaque and was used for analysis in other germplasms. The samples were taken in high-quality quartz cuvette of thickness 2 mm for this measurement. Absorption coefficient (α) values were obtained for each wavelength by using $\alpha = -\ln(T/100)/\text{path-length}$. SPF was calculated from the percentage transmission T obtained at UV wavelengths from 280 to 400 nm using the following equation [5]:

$$\text{SPF} = \frac{\int_{280\text{ nm}}^{400\text{ nm}} E_\lambda S_\lambda \mathrm{d}\lambda}{\int_{280\text{ nm}}^{400\text{ nm}} E_\lambda S_\lambda T_\lambda \mathrm{d}\lambda},$$

where E_λ is the International Commission on Illumination (CIE) erythemal spectral effectiveness, S_λ is the spectral irradiance, and T_λ is the spectral transmittance of the sample. The erythemally weighed UV intensity ($E_\lambda S_\lambda$) values for each wavelength (280–400 nm) were obtained from the combined solar/erythemal response plot as shown in Springsteen et al. [5]. The percentage UV-A and UV-B blocking values were calculated as described by Springsteen et al. [5]. The average transmittance in UV-A (315–400 nm) and UV-B (280–315 nm) regions was obtained, and consequently the percentage blocking for UV-A and

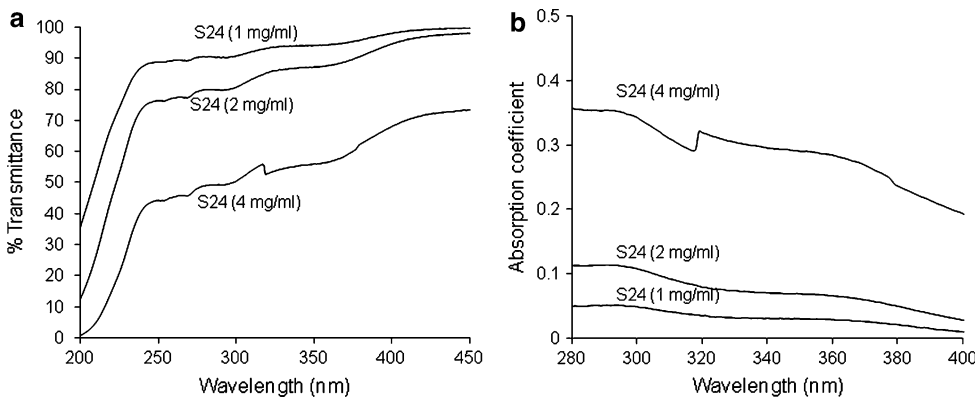

Fig. 1 Percentage transmission (**a**) and absorption coefficient (**b**) of lyophilized gel of the germplasm S24 showing dose dependency

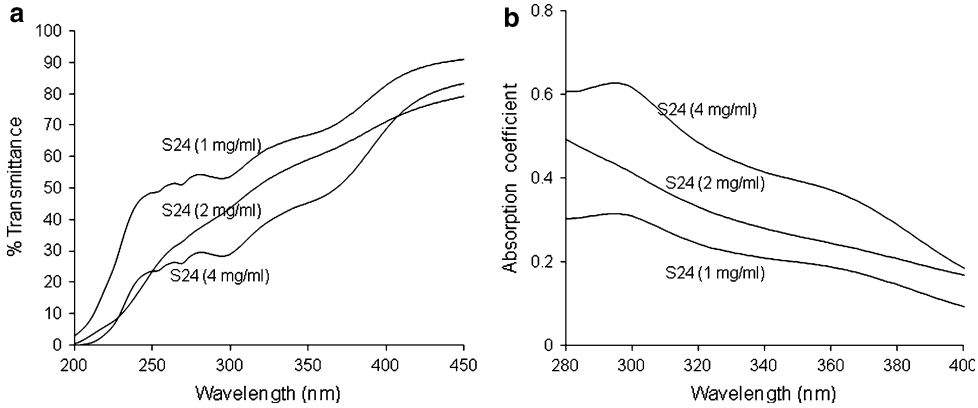

Fig. 2 Percentage transmission (**a**) and absorption coefficient (**b**) of methanolic extracts of the germplasm S24 showing dose dependency

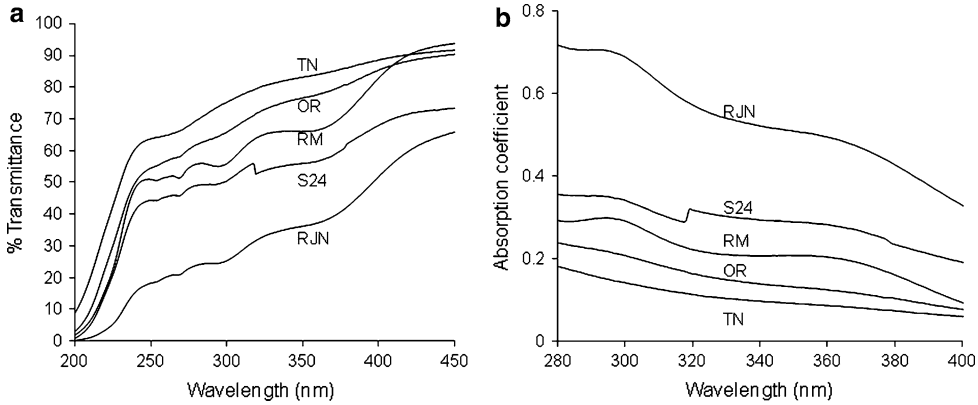

Fig. 3 Percentage transmission (**a**) and absorption coefficient (**b**) of lyophilized gel of *Aloe* germplasms at 4 mg/ml concentration

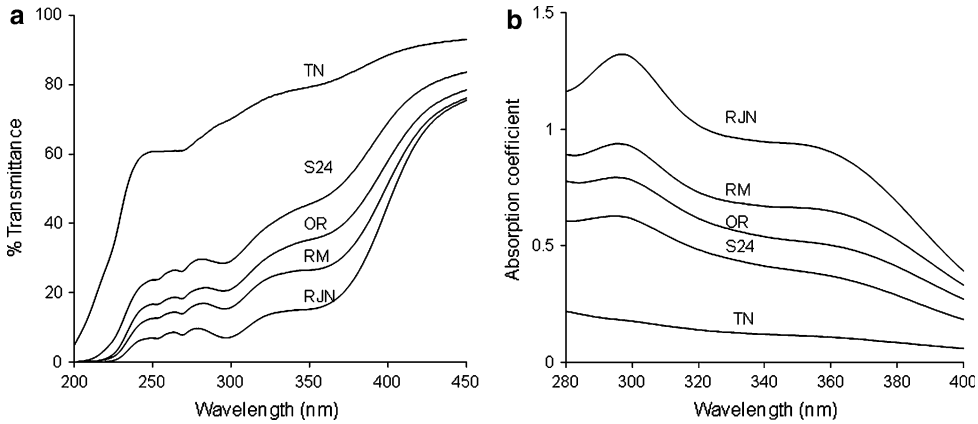

Fig. 4 Percentage transmission (**a**) and absorption coefficient (**b**) of methanolic extracts of *Aloe* germplasms at 4 mg/ml

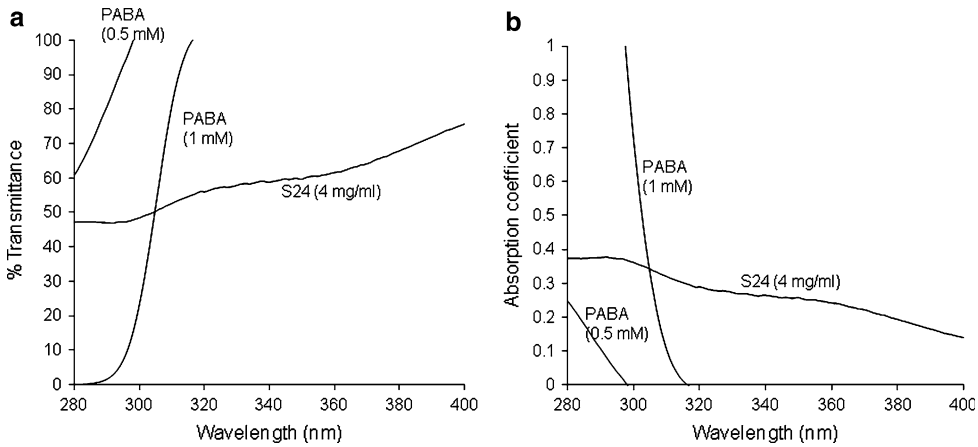

Fig. 5 Percentage transmission (**a**) and absorption coefficient (**b**) of lyophilized gel of S24 and PABA

UV-B were determined. All data are means of three replicated determinations.

Results and discussion

Aloe gel extracts exhibits UV opacity as revealed by percentage transmittance and absorption coefficient in a dose-dependent manner. The coefficient values represent a more reliable presentation of the thickness-independent absorption properties of the gel samples. Among the tested concentrations, 4 mg/ml showed the maximum UV opacity both in lyophilized crude and methanolic extracts (Figs. 1 and 2). Thus this concentration was used to compare the UV opacity potential of various aloe germplasms. UV absorption profiles of various germplasms along with percentage transmittance are presented in Figs. 3 and 4. In all the germplasms UV absorption increased linearly from 400 to 320 nm, which corresponds to the UVA–UVB range. A peak was observed in the 280–320 nm range. A sharp fall in absorbance was observed in the UVC range. These findings suggest a strong UV absorption potential of aloe gel extracts in both the UVA and UVB ranges. Aqueous and alcoholic extracts of black tea also revealed a similar trend in UV absorption profile [17]. However, UVB blocking was more pronounced than UVA. In contrast, para-aminobenzoic acid (PABA), a known sunscreen component, shows only UVB blocking (Fig. 5).

SPF values determined for each germplasm are presented in Fig. 6. The SPF ranged from 1.29 to 3.49 and 1.37 to 9.97 for crude lyophilized and methanolic extracts, respectively. There is a concern about using SPF as it is based on erythema, which incorporates UVB wavelength regions. For a sunscreen, it is also important to have UVA protection. To assess the level of UV protection, we determined the percentage blocking of UVB as well as UVA. Figure 7 summarizes the percentage blocking of UVA and UVB of *Aloe* germplasms. Maximum UVA blocking of 79.12% and UVB blocking of 91.07% was observed in methanolic extracts of RJN. The reference PABA documents SPF of 1.46% and 68.04% UVB blocking at 1 mM concentration. The SPF and UV blocking ability of lyophilized samples followed the trend RJN > S24 > RM > OR > TN, whereas the trend in methanolic extracts noted was RJN > RM > OR > S24 > TN.

Of the tested germplasms, the filtering potential of erythema-causing wavelengths was evidently maximum with RJN and minimum with TN. Compared with lyophilized crude gel, methanolic extract showed higher UV opacity properties, following a similar trend in absorption profiles. This could be due to greater solubility and increased concentrations of gel components in methanolic extract. The use of crude and simple extraction procedures without chromatographic separation of components was found to be an easy approach to screen germplasms with high UV opacity potential. To our knowledge, the present report for the first time describes the UV opacity potential of aloe gel. The variations in UV opacity potential among the germplasms may be due to the presence of different degrees of bioactive components present in *Aloe* gel. Secondary metabolites such as flavonoids and sinapate esters in the plant leaves can absorb in the UV range in

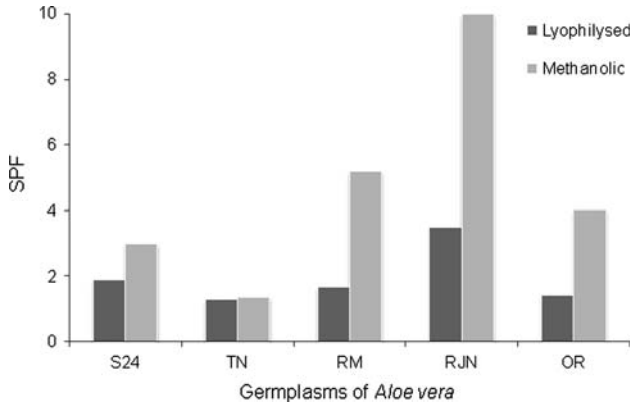

Fig. 6 SPF calculated for lyophilized and methanolic extracts of *Aloe* gel from different gel at 4 mg/ml concentration

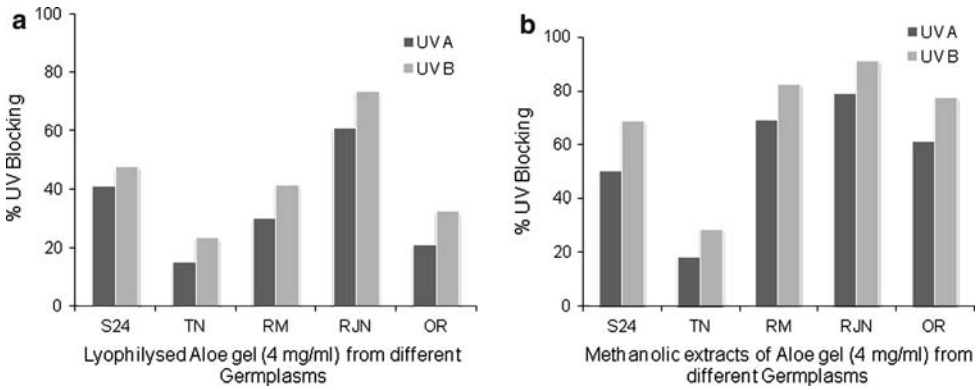

Fig. 7 Percentage UVA and UVB blocking by (**a**) lyophilized and (**b**) methanolic extracts of *Aloe* germplasms at 4 mg/ml concentration

methanolic extracts [18, 19]. Presumably, polyphenols in *Aloe* extracts were the main UV absorbing elements. It has been believed that *Aloe* gel has a modulating effect on the skin by preventing UVB sun rays from sensitizing the skin, especially in the first 24 h following exposure [20]. UV light-filtering power of aloe gel in vivo remains to be investigated.

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