INTRODUCTION

Long term exposure of human skin to solar ultraviolet radiation (UVR) induces the oxidative stress by generating the reactive oxygen species (ROS). ROS triggers skin cancer (Patil, Fegade, Zamindar, & Bhaskar, 2015) and also several skin damages such as sunburn, skin cancer, oxidative stress and also photoaging depending on the amount and form of the UV radiation and on the type of the individual exposed (Mauricio, 1996). Skin is considered as the most exposed part of the body easily affected by direct exposure to the solar radiation including other environmental factors which leads to photodamage (Imam, Azhar, & Mehmoood, 2015).

Based on wavelength UV radiations are classified into three categories namely. UV-A (400nm to 320nm), UV-B (320nm to 280nm) and UV-C (280nm to 100nm). Among these three radiations UV-C is fatal to all living organisms, but luckily blocked by the ozone layer located in the stratosphere. Stratosphere also inhibits UV-B radiations to some extent but due to ozone layer is depletion the rate of UV-B radiation increased; as a result protection from UV-B radiation is acquiring most importance (Ranjithkumar, Sameesh, & Ramakrishnan, 2016). UV-A is capable to penetrate deep in skin induce sun burn or erythema, phototoxic reactions, photoallergy, photosensitivity, Photoaging, skin cancer and immunosuppression. The UV-B radiation causes 1000 times more sun burn than UV-A, and is highly genotoxic (Balakrishnan & Narayanaswamy, 2011). Thus photoprotection is necessary. Photoprotection is achieved by utilizing effective sunscreen and sun protective clothing. Sunscreen products offer sun protection against both UV-A and UV-B radiations. Based on working principle of Sunscreens are classified into two types (i) absorptive and (ii) scattering/reflective. There are many potent physical (Zinc oxide, Titanium oxide) and chemical UV blockers (Oxybenzone, Avobenzone) along with natural substances (like flavonoids and phenolics), which are popularly used for photoprotection. At present sunscreen product contains both chemical and physical agents for sun protection (Gabros & Zito, 2019). Physical and chemical blocker possesses harmful effects as they induce the production of reactive oxygen species (ROS). Whereas, botanical extract based sunscreens with their inherent antioxidant properties help to mitigate the oxidative damages (Kaur & Saraf, 2009) caused by stressors and other factors.

Modern pharmaceutical approach focuses on developing effective formulations based on combination of antioxidative and photoprotective ability of natural products. A large number of Indian herbs possess remarkable antioxidant activities and thus have become focus of intense research of this field (Patra, Pareta, Harwansh, & Kumar, 2011) (Siddiqui, Chowdhury, & Prasad, 2015). In spite of having much literature on antioxidant activity of the selected plant, no absolute data were available

ABSTRACT

UV-B radiation mainly causes photo-oxidative damage resulting DNA damage and skin cancer. Various chemical formulations are available as photoprotective agents. The success of such formulations are either not fully understood or they produce toxicity at certain levels. Hence, herbal formulations with photoprotective activity with promising antioxidant effects are gaining popularity. In the following study, we have investigated methanolic leaf extract of Buchanania lanzan (BMLE) for its photoprotective property with antioxidant activity by different assay methods including FT-IR and HPLC, to identify probable components of photoprotection of BMLE.

KEYWORDS:
Buchanania lanzan, BMLE, SPF, Antioxidant, Quercetin

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INTRODUCTION

Long term exposure of human skin to solar ultraviolet radiation (UVR) induces the oxidative stress by generating the reactive oxygen species (ROS). ROS triggers skin cancer (Patil, Fegade, Zamindar, & Bhaskar, 2015) and also several skin damages such as sunburn, skin cancer, oxidative stress and also photoaging depending on the amount and form of the UV radiation and on the type of the individual exposed (Mauricio, 1996). Skin is considered as the most exposed part of the body easily affected by direct exposure to the solar radiation including other environmental factors which lead to photodamage (Imam, Azhar, & Mehmoood, 2015).

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Modern pharmaceutical approach focuses on developing effective formulations based on combination of antioxidative and photoprotective ability of natural products. A large number of Indian herbs possess remarkable antioxidant activities and thus have become focus of intense research of this field (Patra, Pareta, Harwansh, & Kumar, 2011) (Siddiqui, Chowdhury, & Prasad, 2015). In spite of having much literature on antioxidant activity of the selected plant, no absolute data were available
on SPF. Moreover, there exists confusion about degree of protection offered by topical sunscreens and antioxidants solely on the basis of SPF level (De Galvez, 2010) The efficacy of a sunscreen is generally expressed by Sun Protection Factor (SPF), is the ratio of UV energy required to produce a minimal erythematic dose (MED) in protected skin to unprotected skin.

Recent researches on developing new compounds with photoprotective property includes otcocylene analogues, (Polonini, et al., 2014) bis(indolyl)methane derivatives found in cruciferous plants and marine sources, (Ebrahimzadeh, Hosseinimehr, Hamidinia, & Jafari, 2014) and benzophenone and lactone derivatives (Goncalves, et al., 2018). Quercetin and Rutin incorporated in oil-in-water emulsion shows sun protection factor at a concentration of 10% (w/w). When these two flavonoids were used in association with titanium dioxide the SPF value found to increase to 30 (Choquenet, Couteau, Paparis, & Coiffard, 2008). Furthermore, the photostabilizing and antioxidant properties of quercetin makes it suitable for formulation of broad-spectrum sunscreen having butyl methoxy dibenzoyl methane and octyl methoxy cinnamate (Santo & Matteo, 2010). Goncalves et. al reported a new quercetin derivative (Quercetin 3,7,3',4'-tetraethyl ether) which showed improved skin penetration in sunscreen formulation compared to quercetin (Goncalves, et al., 2019).

This paper attempts to conduct an evaluation of antioxidant and photoprotective property of methanolic leaf extract of *Buchanania lanzan* and also to identify the chemical nature of the major components of BMLE through FT-IR and HPLC analyses.

**MATERIALS AND METHODS**

**Plant material collection and extraction**

Disease free mature leaves of *Buchanania lanzan* were collected from Ausgram (Lat: 23.232437 N Long: 87.863724 E) forest of Burdwan district of West Bengal, India. The leaves were thoroughly washed with running tap water shade dried and ground to a fine powder for extraction in organic solvents. An amount of 5g of leaf dust was taken and defatted using n-hexane and extracted in HPLC grade methanol in soxhlet apparatus at 50°C for 72 hrs. The methanolic leaf extract of *Buchanania lanzan* (BMLE) was filtered using Whatman filter paper no. 1 and concentrated in rotary evaporator and stored in 4°C under desiccation for further experimental studies.

**Quantitative estimation of total Phenol**

Total phenolic content was measured by Folin-ciocalteu’s method (Singleton & Rossi, 1965). For this, 1ml of aliquots and standard gallic acid (10, 20, 40, 60, 80, 100 µg/ml) were kept in test tubes and 10 ml of distilled water and 1ml of Folin-ciocalteu’s reagent (diluted 1:10 with water) were mixed subsequently. After 5 minutes, 10ml of 7% sodium carbonate solution was added and the volume was made upto 25ml. The mixture is then incubated for 90 minutes at room temperature. The intensity of the color was measured at 760nm with spectrophotometer (Lasany). The experiment was performed in triplicates. The blank was prepared using reagent blank with solvent using gallic acid as the standard. The calibration curve was plotted and the total phenol was expressed as mg of gallic acid equivalent weight (GAE)/mg of dry mass.

**Quantitative estimation of total Flavonoid**

Total flavonoid was estimated with the AlCl₃ method (Lamaison & Carnet, 1990) with slight modifications. For this, 1ml BMLE aliquot was added with 4ml of distilled water and 300µl of 5% sodium nitrite. Then 300µl of 10% Aluminium chloride was added to the mixture and 10 minutes incubation period in room temperature was given. After incubation 2ml of 1M NaOH solution added and the volume of the mixture was made upto 10ml with distilled water and the absorbance was measured at 510nm spectrophotometrically. Quercetin was used as the standard.

**Quantitative estimation of total tannin**

Total tannin was estimated according to vanillin hydrochloride method (Burns, 1971). In a test tube to 1ml extract 5ml of vanillin hydrochloride reagent was mixed (which was made just before using by mixing 5ml of 8% hydrochloric acid in methanol with 4% vanillin in methanol keeping in mind that the reagent must not be colored). Then the mixture was incubated in room temperature for 20 minutes followed by measurement of absorbance at 500nm using spectrophotometer. The blank was prepared with vanillin hydrochloride reagent only. The standard curve was prepared using phloroglucinol and the present tannin was calculated as the phloroglucinol equivalent.

**Assessment of Sun Protection Factor**

The SPF factor was measured by the equation of Mansur et. al 1986 (Mansur, Breder, Mansur, & Azulay, 1986). The absorption spectra of BMLE (concentration 500µg/ml) were recorded from the range of 290nm to 320nm in the interval of every 5nm. Three replicates were taken. The equation is as follows

\[
\text{SPF}_{\text{spectrophotometric}} = CF × \frac{\sum_{λ=290}^{320} (EE(λ) \times I(λ) \times \text{Abs}(λ))}{EE} \times I\text{(normalized)}
\]

Where: EE (λ) – erythemal effect spectrum; I (λ) – solar intensity spectrum; Abs (I)- absorbance of sunscreen product ; CF – correction factor (=10). The values of EE x I are constants. They were determined by Sayre *et al.* (1979) (Sayre, Agin, Levee, & Marlowe, 1979).

**Table 1** Normalized product function used in the calculation of SPF

<table>
<thead>
<tr>
<th>Wavelength (Å)</th>
<th>EE x I (normalized)</th>
</tr>
</thead>
<tbody>
<tr>
<td>290</td>
<td>0.0150</td>
</tr>
<tr>
<td>295</td>
<td>0.0817</td>
</tr>
<tr>
<td>300</td>
<td>0.2874</td>
</tr>
<tr>
<td>305</td>
<td>0.3278</td>
</tr>
<tr>
<td>310</td>
<td>0.1864</td>
</tr>
<tr>
<td>315</td>
<td>0.0839</td>
</tr>
<tr>
<td>320</td>
<td>0.0180</td>
</tr>
<tr>
<td>Total</td>
<td>1</td>
</tr>
</tbody>
</table>

EE - erythemal effect spectrum, I - solar intensity spectrum

**Antioxidant activity**

**DPPH radical-scavenging activity**

The DPPH assay of the BMLE was carried out as described previously (Ebrahimzadeh, Hosseinimehr, Hamidinia, & Jafari, 2008). A solution of 0.1 Mm DPPH in methanol was prepared...
and 1.0 mL of this solution was mixed with 1.0 mL of BMLE with concentration 10, 30, 50, 100, 200, 400 µg/mL and commercial antioxidant ascorbic acid. The mixture was left in the dark at 25 °C for 30 min and the absorbance was measured at 517 nm. The ability of BMLE to scavenge DPPH radical was calculated as % inhibition by the following equation:

\[
\text{% Inhibition} = \left( \frac{\text{Absorbance}_{\text{control}} - \text{Absorbance}_{\text{sample}}}{\text{Absorbance}_{\text{control}}} \right) \times 100
\]

Where Absorbance_{control} is the absorbance of the DPPH radical + methanol; Absorbance_{sample} is the absorbance of DPPH radical + BMLE or ascorbic acid. IC₅₀ values denote the concentration of the sample or standard, which is required to scavenge 50% of DPPH free radicals.

Reducing power determination

The reducing power of BMLE was estimated according to Bhalodia et al., 2013 (Bhalodia, Nariya, Acharya, & Shukla, 2013). 1 ml of BMLE aliquots (10 – 100 µg/ml) were mixed with 2.5 ml phosphate buffer (pH6.6) and 2.5 ml of 1% potassium ferricyanide [K₃Fe(CN)₆]. It was then incubated at 50°C for 20 min. After incubation the reaction was stopped by adding 10% 2.5ml (w/v) of trichloroacetic acid to it and centrifuged at 3000 rpm for 10 minutes. The supernatant was mixed with 2.5 ml distilled water and 0.5 ml, 0.1% ferric chloride. The absorbance of the mixture was taken at 700 nm. Ascorbic acid was kept as positive control.

ABTS free radical assay

In the ABTS radical cation decolorization assay was measured by the method of Re et al, 1999 (Re, Pellegrini, Proteggente, Pannala, Yang, & Rice-Evans, 1999). The ABTS⁺ cation was generated by oxidation of 7 mM ABTS solution with 2.45 mM potassium persulfate solution in equal volume. This mixture was kept in dark for 12-16 hrs at 25°C for reaction. The reaction mixture was then diluted with concentrated methanol until the absorbance (734 nm) reached 0.700 ± 0.001 value. 1 µL of this solution was added to the (100-1000 µg/ml) of BMLE and ascorbic acid and absorbance measured at 734 nm. The reducing power of BMLE was estimated according to the above mentioned protocols. The results thus obtained are detailed below.

### RESULTS AND DISCUSSION

#### Quantification of Phenol, Flavonoid and Tannin

Quantitative estimation of three important phytometabolites present in BMLE namely phenol, flavonoid and tannin were carried out according to the above mentioned protocols. The results thus obtained are detailed below-

<table>
<thead>
<tr>
<th>Phenolic contents</th>
<th>Amount (µg standard equivalent/mg of extract powder)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenol</td>
<td>97.50±0.953</td>
</tr>
<tr>
<td>Flavonoid</td>
<td>71.33±1.630</td>
</tr>
<tr>
<td>Tannin</td>
<td>106.85±1.519</td>
</tr>
</tbody>
</table>

The BMLE contains total phenol 97.5±0.953 µg gallic acid equivalent/ mg of extract powder by reference to standard curve (y = 0.002x +0.090, R² = 0.991). The total flavonoid content was 71.33 ± 1.630 µg quercetin equivalent/mg of extract powder by reference to standard curve (y = 0.003x – 0.009, R² = 0.982) and tannin content was 106.85±1.519 µg phloroglucinol equivalent/ mg of extract by reference to standard curve (y = 0.010x + 0.005, R² = 0.997).

#### Estimation of Sun Protection Factor

The absorbance of BMLE (500 µg/ml) from 290nm to 320nm showed the following SPF value.

<table>
<thead>
<tr>
<th>Wave Length (λ, nm)</th>
<th>EE (λ) X I (λ) employed</th>
<th>Absorbance (A)</th>
<th>EE X I X A</th>
<th>SPF = [\Sigma EE(λ) / (I(λ) × X × X)]</th>
</tr>
</thead>
<tbody>
<tr>
<td>290</td>
<td>0.0150</td>
<td>1.318 ± 0.009</td>
<td>0.0198 ± 0.0015</td>
<td>12.725± 0.083</td>
</tr>
<tr>
<td>295</td>
<td>0.0817</td>
<td>1.274 ± 0.007</td>
<td>0.1041 ± 0.0060</td>
<td></td>
</tr>
<tr>
<td>300</td>
<td>0.2874</td>
<td>1.265 ± 0.006</td>
<td>0.3636 ± 0.0015</td>
<td></td>
</tr>
<tr>
<td>305</td>
<td>0.3278</td>
<td>1.266 ± 0.009</td>
<td>0.4147 ± 0.0031</td>
<td></td>
</tr>
<tr>
<td>310</td>
<td>0.1864</td>
<td>1.277 ± 0.008</td>
<td>0.2381 ± 0.0016</td>
<td></td>
</tr>
<tr>
<td>315</td>
<td>0.0837</td>
<td>1.296 ± 0.009</td>
<td>0.1085 ± 0.00080</td>
<td></td>
</tr>
<tr>
<td>320</td>
<td>0.0180</td>
<td>1.324 ± 0.012</td>
<td>0.0238 ± 0.00025</td>
<td></td>
</tr>
</tbody>
</table>

The presence of so many amounts of flavonoids helps the extract to absorb UV radiation and gives it the SPF value of 12.725 ± 0.083, which can effectively block 90-93% of UVR (according to Graph1).
Antioxidant Assay

**DPPH Assay**

Graph 1: Ultraviolet B protection level by sun protection factor (SPF) (Dale Wilson, Moon, & Armstrong, 2012)

Graph 2: Plot showing DPPH scavenging activity of Ascorbic acid and BMLE and the bar graph represents the IC<sub>50</sub> value of DPPH scavenging activity of ascorbic acid and BMLE to be 5.33 and 50.35 respectively.

**Reducing Power Assay**

Graph 3: Plot showing Reducing power of Ascorbic acid and BMLE and the bar graph represents the IC<sub>50</sub> value of reducing power of ascorbic acid and BMLE to be 16.52 and 97.66 respectively.

**ABTS scavenging Assay**

Graph 4: Plot showing ABTS scavenging activity of Ascorbic acid and BMLE and the bar graph represents the IC<sub>50</sub> value of ABTS scavenging activity of ascorbic acid and BMLE to be 25.71 and 336.06 respectively.

SPF = 12.725±0.083

ABC
damage and skin cancer (Strack, 1997). Antioxidant activity also helps in UV protection (Kittiswannahach, Borisut, Wanasawas, Ponpanich, Rattanasuk, & Chulasiri, 2008). It is reported that high SPF values in Dracocephalum moldavica and Viola tricolor (24.79 and 25.69 respectively) may be due to their high phenolic contents (Khazaeli & Mehrabani, 2008). Flavonoids are considered as the main group of antioxidant in Halimium halimifolium (Rebaya, et al., 2014). Quercetin, particularly Quercitrin (glycosylated form of Quercetin) effectively inhibits UV-B radiation induced oxidative damage both under in vivo and in vitro conditions. Appreciable reduction of UV-B induced ROS generation in JB6 cell lines has been reported. The mechanism involved restoration of catalase expression and GSH/GSSG ratio upon UV exposure, which minimized oxidative DNA damage apoptosis and skin inflammation. Thus Quercetin plays an important role not only as photoprotectant but also a potent antioxidant (Yin, et al., 2013). Earlier report claims that quercetin can also alleviate the harmful effects of UV-A radiation (Inal, Kahraman, & Koken, 2001).

CONCLUSION

From this study it can be concluded that, the methanolic leaf extract of Buchanania lanzan is an effective photoprotectant and can block 90-93% of UV-B radiation. Moreover, it possesses a significant amount of antioxidant activity. So, the leaf extract with high amount of quercitin can be used in different pharmaceutical formulations as potent anti-photoaging skin cream. Complete extraction, isolation and purification of specific phytometabolites endowed with UV-induced photoaging preventive activities need to be examined in detail before commercial application.

Acknowledgement

I hereby acknowledge The Head, Department of Botany, University of Burdwan for providing me infrastructural support and also recognize The Head, Department of Biotechnology, University of Burdwan for HPLC facility.

References


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