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Research Article

In vitro assessment of Sun Protection Factor and antioxidant activities of certain phytoextracts

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Abstract

The topical application of sunscreens is indispensable for preventing the effects of UV rays, which range from tanning to possible carcinogenesis. Recent reports indicate that residues of important commercially available UV filters are detected in ecosystem and in users. There are great concerns over the possibility of ecological disturbances caused by UV filters. This is particularly true in the case of reported coral reef bleaching. Further, a few reports indicate that some UV filters may have adverse health effects. In view of this, the present study was carried out to determine sun protection factor, total phenolics, radical scavenging activity and total antioxidant capacity of star anise pods, fennel and flax seeds and moringa leaves. Methanolic extracts of the said plant materials were prepared and concentrated under vacuum. In vitro assays were performed to determine the sun protection factor as well as the antioxidant potential of methanolic extracts. In vitro SPF activity and total phenolics were highest in the case of moringa leaf extract, which also exhibited appreciable antioxidant activity as reflected by radical scavenging activity and total antioxidant capacity. Our data indicate that moringa leaf extract, with its higher SPF and antioxidant activity, could be further explored for incorporation into herbal sunscreen formulations.

Keywords: Fennel seeds, Flax seeds, Moringa leaves, Star Anise pods, Sun Protection Factor, Antioxidant capacity

Introduction

Ultraviolet radiation represents part of electromagnetic spectrum ranging from 40-400nm wavelength. This type of electromagnetic (EM) radiation is majorly emitted by sun. The region of UVRs are known to be divided into vacuum UV (40-190 nm), followed by far UV (190-220 nm), post which are UVC, UVB and UVA with wavelength ranging between 220-290 nm, 290-320 nm and 320-400 nm respectively [1–3]. The UVR emitted by the sunlight reaching the earth's surface are usually composed of approximately 2-5 % UVB and 95-98% UVA and are mostly devoid of UVC since they are absorbed by the atoms of ozone in the earth's stratosphere (Mishra et al., 2011; United Nations Environment Programme, 1979). UVB due to its shorter wavelength are mostly responsible for epidermal damage but UVA due to its longer wavelength has remarkable penetration capacity thereby causing DNA damage and various tissue damage[3,5,6]. UVRs are required for synthesis of vitamin D from pro-vitamin D [7,8], and inadequate UVB exposure is positively linked to the development of rickets and osteoporosis in elderly population [7–9]. However, chronic exposure to UVR can potentially lead to damage of skin, immune system and eyes [2]. Acute excess exposure to UVR will first lead to severe sunburn whereas, chronic exposure can lead to photo-aging, DNA damage thereby leading to various melanoma conditions like xeroderma pigmentosum, trichodiodystropy and Cockaye's syndrome [1,2,7,8]. Further, UV exposure can lead to various pre-malignant cutaneous conditions such as solar keratosis, squamous cell carcinoma and malignant melanoma. Acute intermittent exposure to UVR during youth can increase the incidence of cancer in lip, ocular melanoma, cataracts, age-related macular degeneration (AMD) [2,5,6,8,10]

Sunscreens used for external photo-protection contain active photo-protective ingredient which can be either of physical or chemical nature. These in principle act by absorbing, scattering or reflecting the UV rays [3,7,11,12]. The sun protection factor is defined as the ratio of the minimum dose of ultraviolet energy needed to elicit a minimal erythema on skin protected by sunscreen application to the dose of energy required to produce the same erythema on unprotected skin [13]. An SPF of 15 correlates with 93.3 percent of UVB absorption, whereas SPF 30 correlates with 96.7 percent, SPF 45 correlates with 97.8 percent, and SPF 50 correlates with 98 percent UVB absorption. The formula to calculate sunscreen percentage absorption based on SPF is: absorption = 100 – (100/SPF) [7,12]. Ideally sunscreens should be safe, chemically inert, non- toxic to both humans and the environment, most importantly photo-stable along with providing complete protection against UVR damage. In the quest for the same, plant-derived products may serve as excellent photo-protective agents. Further, other properties like anti-bacterial and antioxidant attributes may enhance the value of phytconstituents as sunscreens. This study was carried out to screen commonly consumed plant substances for their sun protection factor and antioxidant activities *in vitro*.

Materials and Methods

Chemicals

Ammonium molybdate, ascorbic acid, disodium hydrogen phosphate, FC reagent, gallic acid, hexane, methanol and sodium bicarbonate were obtained from Sisco Research Laboratories (India). Star anise pods, fennel and flax seeds and moringa leaves were purchased from local market. All other chemicals used in the present study were of analytical grade.

Extraction

Dried and powdered plant materials were extracted in 10 volumes of methanol. The extract was filtered and the filtrate was concentrated under vacuum to obtain the oleoresins [14].

Assays

In vitro sun protection factor

Sun protection factor of extracts (0.1mg/mL dissolved in methanol) was determined by recording their absorbencies between 290-320nm. The SPF was determined by the Mansur equation as described earlier [15].

Total Polyphenol

The phenolics in the extract reduces the reagent to produce molybdenum-tungsten blue which is read at 765nm [16].

Total antioxidant activity (TAC)

The total antioxidant capacity (TAC) of the test extract was assessed using the phosphomolybdenum method which is based on the reduction of Mo (VI) to Mo (V) complex under acidic conditions as described earlier [17].

Radical scavenging activity

The radical scavenging activity exhibited by standard (ascorbic acid) or extracts was determined by monitoring scavenging of the free radical α, α-diphenyl-β-picrylhydrazyl (DPPH) spectrophotometrically at 514nm [18].

UV-VIS spectrum of Moringa leaf extract

The UV-Vis absorption spectrum of moringa leaf extract was studied at a concentration of 0.1mg/mL using a double beam spectrophotometer against methanol blank by measring the absorbancies from 200 to 800nm.

Statistical Analysis

Data have been represented as Mean ± SD (n=3). ANOVA and Tukey post hoc tests were determined to determine the significances in differences of means.

Results and Discussion

Data on *in vitro* sun protection factor of different extracts are depicted in **Figure 1**. Moringa leaf (MLE) extract was found to exhibit highest SPF activity followed by star anise extract (SAE), fennel seed extract (FnE) and flax seed extract (FxE). When tested for scavenging of DPPH radical, ascorbic acid exhibited an EC50 value of 31.3±0.3μg/mL (data not shown). Among the extracts tested, MLE was most potent followed by FnE. The radical scavenging activities of FxE and SAE were comparable and lower than that of FnE (**Figure 2**). Analysis of molybdenum reduction potency revealed that FnE exhibited highest TAC. The potencies of SAE and MLE were comparable and only slightly but statistically lower than FnE (**Figure 3**). Total phenolics content was highest in MLE, while no significant difference was found between FnE, FxE and SAE for total phenolics content (**Figure 4**). Since moringa leaf extract exhibited highest SPF in among the phytoextracts studied in the present investigation, we recorded the UV-Vis absorption spectrum of the extract (**Figure 5).** The extract exhibited appreciable absorbance in the UV range with an absorbance of 0.48 at 268nm.

Sunscreen are vital for topical protection against UVR the chemicals used in commercial non-herbal sunscreen contain cinnamates, benzophenones, dibenzoylmethanes and anthralates as organic UV filters or zinc oxide or titanium oxide as inorganic UV filters [19]. However, UV filters have come under scrutiny for their environmental effects and toxicity. oxybenzone, octocrylene, octinoxate, and ethylhexyl salicylate are ubiquitously found in water bodies around the world [20]. Danovaro et al suggested that sunscreen could cause coral bleaching [21]. Astonishingly, 97% of tested individuals were found to contain oxybenzone in urine samples [22]. A study measuring the effect on pS2-gene transcription in MCF-7 cells has reported that many UV filters possess estrogenic activity [23]. Oxybenzone has been reported to elicit decrease in chlorophyll fluorescence, genotoxicity and ossification in coral [24]. Schlumpf et al reported that UV filters exhibit estrogenic and anti-androgenic activity [20]. Similarly, a study utilizing 14-day juvenile rainbow trout assay for plasma vitellogenin also demonstrated that oxybenzone exerts estrogenic activity [25]. Dermal application of benzophenone-2 has been reported to be associated with enhanced proliferation of splenocytes and hyperthyroidism in rats [26]. Further, benzophenone-3 is also known for allergenicity [27]. While environmental relevance of concentrations at which sunscreens are tested is debated, it is clear that many sunscreens are of concern for effects on health and ecosystem.

Owing to their chromophoric properties, plant products are receiving increasing attention as possible active ingredients in sunscreens. Plant-based sunscreens offer advantage of incorporating actives which are resourced from renewable sources, with no deleterious side effects both on the environment and humans [28–33]. Plant extract contains wide range of phytoactives such as phenolics, saponins, alkaloids and terpenoids [34]. Phytoactives such as flavonoids, polyphenolic acid and high molecular weight polyphenols acts as excellent source for photo-protection due to UV absorption behavior [34,35]. Thus it is not surprising that many investigators have explored herbal bioactives as actives for formulating sunscreens [33,36–38]. In the present study, we observed that MLE exhibited highest SPF and strongest radical scavenging activity.

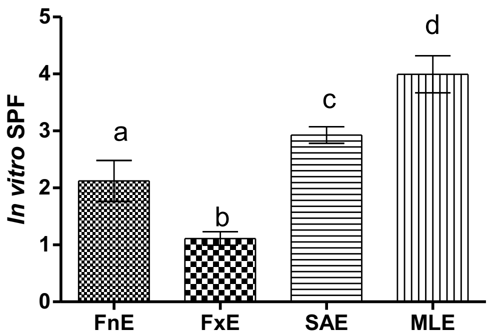

Figure 1. *In vitro sun protection factor (SPF) of fennel seed extract (FnE), flax seed extract (FxE), star anise extract (SAE) and moringa leaf extract (MLE). Data presented as mean ± SD (n=3); Data analyzed by ANOVA and Tukey test, bars with different letters are significantly different (p< 0.05)*

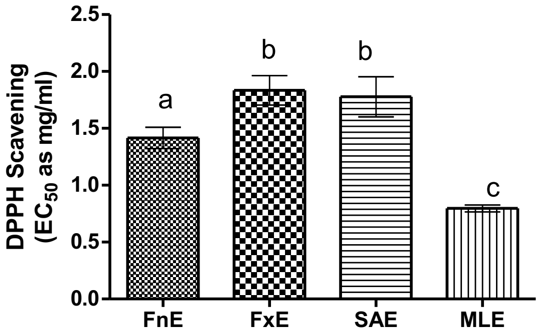

Figure 2. *DPPH radical scavenging activity of fennel seed extract (FnE), flax seed extract (FxE), star anise extract (SAE) and moringa leaf extract (MLE). Data presented as mean ± SD (n=3); Data analyzed by ANOVA and Tukey test, bars with different letters are significantly different (p< 0.05)*

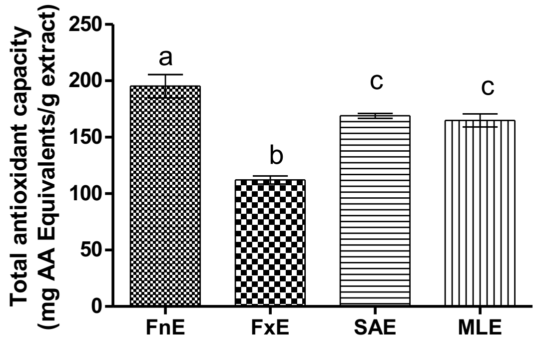

Figure 3. *Total antioxidant capacity of fennel seed extract (FnE), flax seed extract (FxE), star anise extract (SAE) and moringa leaf extract (MLE). Data presented as mean ± SD (n=3); Data analyzed by ANOVA and Tukey test, bars with different letters are significantly different (p< 0.05)*

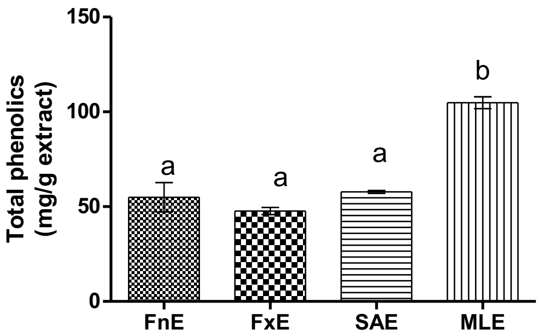

Figure 4. *Total phenolics content of fennel seed extract (FnE), flax seed extract (FxE), star anise extract (SAE) and moringa leaf extract (MLE). Data presented as mean ± SD (n=3); Data analyzed by ANOVA and Tukey test, bars with different letters are significantly different (p< 0.05)*

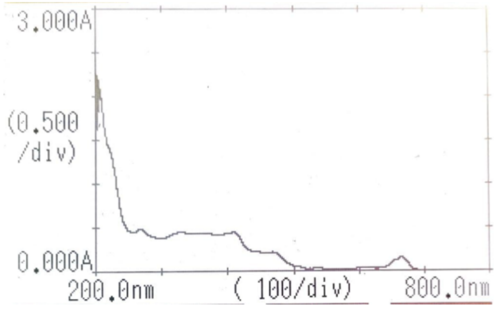

Figure 5. *Representative UV-VIS spectrum of moringa leaf extract.*

Conclusion

The presented study was undertaken to screen plant materials, with known history of consumption by human beings, for in vitro sun protection activity and antioxidant activity. The study was conducted with methanolic extracts of fennel seeds, star anise pods, flax seeds and moringa leaf. Our data revealed that morning leaf extract, among the tested phytoextracts, possesses high SPF activity (in vitro), total phenolics, DPPH scavenging activity and appreciable total antioxidant capacity. Based on the data, we opine that MLE can be further explored as an active ingredient in plant based sun protection formulations

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