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Research Article

In vitro sun screening activity of Sri Lankan orthodox black tea (*Camellia Sinensis* linn)

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ABSTRACT

Currently, there is demand for the development of herbal sunscreen formulations to suppress harmful effects of UV rays. In this regard, this study, was conducted to investigate the sun screen potential of Sri Lankan Orthodox black tea (made from buds and top most leaves of *Camellia sinensis* L plant) using three grades (Dust No:1, Broken Orange Pekoe and Orange Pekoe) using UV spectroscopic technique and Mansur equation. Sun Protection Factor (SPF) value was determined using 20% aqueous extracts (Black tea brews). The results revealed that all three tea samples had markedly high absorbance values (1.4 to 4.2) at 290-320 nm range and SPF values above 15 which are considered as the threshold value for good sunscreen. The SPF value of Dust No:1, B.O.P.F and O.P were respectively 36, 23 and 22. This is a novel finding for Sri Lankan black tea. It is concluded that Sri Lankan black tea, especially, Dust No: 1 can function as an efficient sunscreen agent and has great promise to be developed as cheap, safe and effective topical botanical sunscreen acting via multiple mechanisms (considering its other reported bioactivities).

Key words: Black tea, Dust No: 1, Broken Orange Pekoe, Orange Pekoe, sunscreen, photo-protection, sun protection factor, *Camellia sinensis*

INTRODUCTION

The earth is continuously bombarded with ultraviolet rays (UV-R) from the sun, which are harmful to human skin, the outermost covering of the body, and hair [1]. Human skin and hair are very susceptible target organs to UV radiation [1]. There are three main categories of UVRs depending on their wave length;: UVC (200-280nm), UVB (280-320 nm) and UVA (320-400 nm)[1,2,3]. Of these, over exposure of UVB is mainly responsible for causing deleterious effects on the skin, such as sunburn, erythrema, inflammation, hyperpigmentation (tanning), rough texture, wrinkling, hyperplasia, local immunosupression , genotoxicity, photoageing, photocarcinogenesis [1,2,4,5,6]. In this context, it is of interest to note that UVB rays are primary cause of skin cancer [1,2,7]; the most common human cancer is skin cancer [8] and more than one million new cases of skin cancer is documented each year in United States of America alone [6]. However, luckily, skin cancer is almost fully curable if treated before it has a chance to spread [7,8].

Dermatologists, strongly recommend using a sunscreen (a photoprotective) with a sun protection factor (SPF) value of 15 or grater preferably year around to protect the skin against harmful UV rays, especially, the UVB [7,8].Currently, in the market several topical sunscreen formulations are available both synthetic and herbal in the form of creams, lotions, oils, gels, sprays to be applied on to the skin [1,2,3,6,9]. The use of synthetic sunscreens appears to be limited as there is a risk of developing contact and/or an irritant dermatitis, hypersensitivity, allergies and even melanomas and skin cancers [6,10,11]. Also, their safety following long term use is not established [10,11]. Further, these are capable of interfering only with certain selected pathways involved in skin carcinogenesis [3,9,10] and often these stain clothings [7]. On the other hand, herbal sunscreens are claimed to be

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relatively safe, devoid of undesirable side effects and often has the potential to impair skin cancer formation by disrupting multiple pathways which are involved in carcinogenesis [9,10].

Tea brew made from *Camellia sinensis* L. (Family: Theaceae) is commonly used as a home remedy for sunburns [10]. And, now several laboratory studies confirm that tea extracts or their ingredients, especially, green tea, taken topically or orally protect the skin from hazardous effects of suns UV rays [9,10,11,12,13]. As a result, currently there is an emerging trend to incorporate tea (both green and black) or its constituents to sunscreen formulations [9,10]. However, it is known that bioactivity of black tea varies with the country of origin, techniques of manufacture, particle size, grade of tea, agro climatic elevations amongst other things [14,15]. Hence, we thought it is logical to investigate the sunscreen potential of Sri Lankan orthodox black tea which has not been tested so far. Sri Lanka is currently the main producer and exporter of orthodox back tea [16]. Therefore, this study was initiated to investigate the sunscreen potential of three grades of Sri Lankan orthodox back tea, namely, Dust No:1, Broken Orange Pekoe Fannings (B.O.P.F) and Orange Pekoe (O.P) by evaluating SPF values employing an UV spectroscopic technique and Mansur equation as described previously by several authors.[3,5,9,13]

MATERIAL AND METHODS

Sources of tea; Black tea samples used in this study were manufactured using orthodox rotovane technique with fresh two or three top most tender leaves and buds of *C. sinensis* harvested from three different tea estates belonging to three different agro climetic elevations; Dust No: 1 from St. Coombs estate of Tea Research Institute, Talawakele, Sri Lanka (1382 m above mean sea level; high grown) B.O.P.F form Dotaluoya estate, Kegalle, Sri Lanka (120 m above mean sea level; mid grown) and O.P from Jochims tea estate of the Tea Research Institute Hedallana, Ratnapura, Sri Lanka(29 m above mean sea level; lower grown)

Seive analysis: The composition of true to size particles define for each grade of tea samples was determine in triplicate according to Samaraweera [17]) using a sieve shaker(Retsah AS 200, Retsch, Gmbh, Hann, Germany) fixed with a standared set of sieve.

Organoleptic analysis: Typical characters (leaf characteristics, infuse leaf characteristics and liquor characteristics) of each tea sample belong in to different agro climetic elevation were organoleptically asscessed by expert professional tea tasters of the Tea tasting unit Sri Lanka Tea Board Kolpitty, Sri Lanka. The tea samples were then packed in triple laminated aluminium foil bags (1 kg each) and stored at-20 C^0 until use.

Preparation of Black tea brews: BTBs were made separately accordingly ISO stranded (ISO 3103) by adding 2 g of each grade of tea 100 ml of boiling water and brewing for 5 minutes[18]. Then the BTBs squeezed through a muscling cloth and diluted to 20% using water for the evaluation of sun protection factors (SPF) values.

***In vitro* evaluation of sun protection factor of different BTBs**: *In vitro* SPF values of 20 % BTBs of Dust No: 1, B.O.P.F, and O.P were determine using a UV- VIS Spectrophotometer (Genesys 10 S , Thermo Fisher Scientific, Madison, USA) as described previously in detail by several investigators. [3,5,9,13] Briefly, absorbance of UV radiations through each aliquot of BTB infusion in quartz cell were determine in triplicate from 290 to 320 nm, at 5 minutes intervals taking distilled water as the blank. SPF values were then determined using the Mansur equation [3,5,9,13] given below.

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

Where EE (i)- erythemal effect spectrum; I(I)- solar intensity spectrum,; Abs- Absorbance of sunscreen product; CF- correction factor (=10). The value of EE X I are constant and predetermined [3].

Statistical analysis: The results are represented as mean ±SEM. Stastical comparisons were made using one-way ANOVA with Tukeys Family Error post hoc test. Significance was set at $P<0.05$.

RESULTS

The results of the sieve analysis reveled that all the three tea samples had more than 80% tea particles falling within the range characteristic for each grade. (17); Dust No:1(range ; 300-500 µm), B.O.P.F (range; 500-800 µm) and O.P (range; 1400-2000 µm).

Organoleptic assessment of black tea sample revealed that their leaf characteristics, infused leaf characteristics and liquor characteristics can be accepted as typical to high grown Dust No: 1, mid grown B.O.P.F. and lower grown O.P. grades of Sri Lankan black tea.

The absorbance values obtained for the three grades of black tea (Dust No:1, B.O.P.F and O.P) is depicted in Table 1. As shown, all the three grades

of black tea showed markedly high absorbance values (ranging from 1.4 to 4.2).

The mean computed SPF values for the three grades of black tea are shown in Table 2. The highest SPF value was evident with Dust No: 1 whilst, the other two grades (B.O.P.F and O.P) had almost identical SPF values. This difference in SPF values of Dust No: 1 was significantly ($P<0.05$) different to that of B.O.P.F and O.P. Interestingly , all the three SPF values were higher than 15 which is the threshold values recommended for a good sunscreen formulation [7,8].

DISCUSSION

This study examined the *in vitro* sunscreening potential (in terms of SPF values) of three grades (Dust No:1, B.O.P.F and O.P) of Sri Lankan orthodox black tea using UV spectroscopic technique and Mansur equation [3,5,9,13]. The SPF value is a worldwide standard for assessing the effectiveness and efficacy of sunscreen products[2,3,5,7,8,9,13]. The technique used is well accepted, simple, inexpensive and generate quick results which are valid, reliable and reproducible [2,3,4,5,6,9]. Also, it avoids ethical issues associated with *in viv*o testing [2,12]. Nevertheless, it should be pointed out that amoungst the several *in vitro* techniques currently available to determine SPF values, none is universally accepted [6,9].

The tea samples used in this study were factory fresh, unblended and typical for the grade and agro climatic elevation (in terms of sieve analysis and orgonoleptic properties) and the tea brews were made according to ISO specifications [19] unlike in previous studies where these crucial information are poorly documented [7,9,10,11,12,13]. Moreover, in this study, a 20% aqueous extracts (infusions) were used in evaluating the SPF values as has been done in previous studies [3,4,6,9]. Hence, the results obtained are valid and can be meaningfully interpreted.

The results unequivocally demonstrate that 20% aqueous brews of Sri Lankan orthodox black tea tested (Dust No: 1, B.O.P.F and O.P) possess marked sunscreen activity. This activity was remarkably high with Dust No: 1 which had a value of 36. The SPF values of B.O.P.F and O.P were almost identical, but was significantly lower than that of Dust No: 1. (by 64%). Importantly, the SPF values of all the three grades of tea samples tested were higher than 15: dermatologists strongly recommend to topically apply sunscreens having SPF value of 15 or more to avoid harmful effects of UV rays [7,8]. Further, high absorbance values were evident in all three tea grades at 290-320 nm wave length range of UV rays which is also a desirable character of a potent sunscreen [13]. Taken together, these results show the Sri Lankan Orthodox black teas have great promise to be developed as potent topical sunscreen. This promise is further strengthen as Sri Lankan black tea can be also consumed regularly as an oral supplement to provide base-line protection from UV rays encountered in daily life and those reflected from surfaces such as concrete surfaces of tall building. Nowadays, use of oral antioxidant supplements for this purpose is encouraged by clinicians [1,9].

We have shown that all the black tea samples contained a variety of flavonoids (such as epigallocatechingallate, epigallocatechin, epicatechingallate, epicatechin, gallocatechin and catechin) and polyphenoles (theaflavins and thearubigins) caffine (alkaloid) and theanine (a unique amino acid in tea) [20,21,22]. However, the composition of these phytoconstituents was different [20,21,22]. Such compositional differences in phytoconstituents of black tea are also reported by others [23].This difference may be attributed to the difference in potencies in sunscreening action between tea samples evident in this study:Dust No:1> B.O.P.F and O.P. It can also result from variations in extraction of above mentioned water soluble phytoconstituents from the tea samples due to their dissimilarities of particle sizes [19]. Alternatively,this difference is potency in sunscreening action may be due to differences in altitude and climate from where the tea samples originated. Dust No: 1 and B.O.P.F were from high grown elevation while the O.P was from low grown elevation. Interestingly, potency difference between Sri Lankan black grades has been reported previously for tea induced diuresis [24,25,26] and blood anti clotting activity [27].

Now, it is well established that UVB rays absorbed by the skin causes the production of aggressive free radicals such as ($O_2^{\cdot-}$, $^1O_2$, H_2O_2, $^{\cdot}OH$, $ROO^{\cdot}$.[1,4,5,10,11] It has also been reported that irradiation with UV rays produces a decrease in levels of antioxidants, deactivation of anti oxidenets enzymes (super oxide dis mutase, caterlase and glutathione peroxydase) and an increase in tha markers of lipid peroxidation ($L^{\cdot}$, $LO^{\cdot}$, $LOO^{\cdot}$) [1]. Free radicals are linked with inflammation, accelerated ageing and skin cancers [1,4,5,11,12]. Black tea including Sri Lankan grades [23,28,] and its phytoconstituents are shown to function as powerful antioxidants [23,28,29]. In fact, black tea is one of the strongest herbal antioxidants sreported so far [23]. Further, thearubigins and theaflavins of black tea are known

to inhibit lipid peroxidation [12], Since incorporation of antioxidants is now widely recommended in sunscreens, [1], the presence of efficient free radical scavenging activity in Sri Lankan black tea is another positive factor for it to be formulated into sunscreens. Sri Lankan black teas possess marked *in vivo* anti inflammatory activity [30]. which is regarded as a desirable character to be present in a sunscreen. Accumulation of Advance glycation end products (AGEs) is implicated as a causative factor of premature ageing [5] and Sri Lankan black tea has been shown to have profound antiglycation and glycation crossbridge breaking activities *in vitro* [31]. Obviously, presence of these two activities in Sri Lankan black tea would further enhance it is value substantially as a potential sunscreen. Interestingly, tea polyphenols and theoflavins are reported to be present in the prostate of mice and humans following black tea consumption [12]. And both theaflavins and therabuginshave been shown to prevent human epidermal carcinoma and human malignant cell proliferation through apoptosis [12]. What is more, is that tannic acid theobromin in tea are claimed to facilitate removal of heat from sunburns [8]. Presence of phytoscreening action together with antioxidant, antiinflammatory, anti AGEs activity (both antiglycation and cross linker breaking) and anticancer activities undisputedly makes Sri Lankan black tea to be an outstanding safe wide spectrum sunscreen. It is very rarely such several important multiple biological activities are present in a single herbal extract.

CONCLUTION

This study shows, for the first time, that Sri Lankan Orthodox black tea, namely Dust No:1, B.O.P.F and O.P possess profound *in vitro* sunscreen (photo protection) activity and Sri Lankan black tea has a huge potential to be developed as a cheap and safe wide spectrum sunscreen formulation acting via multiple mechanisms.

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Table 1: Absorbance of 20% aqueous Dust No:1,Broken Orange Pekoe (B.O.P.F) and Orange Pekoe (O.P) grades of Sri Lankan black tea

Wavelength(nm)	EE x I	Dust grade No: 1	B.O.P.F.	O.P.
290	0.0150	4.298 ± 0.006	3.435 ± 0.001	3.691 ± 0.004
295	0.0817	4.287 ± 0.005	2.879 ± 0.001	3.012 ± 0.005
300	0.2874	3.947 ± 0.004	2.498 ± 0.003	2.512 ± 0.003
305	0.3278	3.558 ± 0.005	2.220 ± 0.005	2.155 ± 0.005
310	0.1864	3.159 ± 0.003	1.985 ± 0.003	1.850 ± 0.001
315	0.0837	2.798 ± 0.001	1.777 ± 0.001	1.591 ± 0.003
320	0.0180	2.513 ± 0.001	1.625 ± 0.002	1.400 ± 0.003

EE-Erythemal effect spectrum: I-solar intensity spectrum

Table 2: Sun protection factor (SPF) of 20% aqueous Dust No:1 Broken Orange Pekoe (B.O.P.F) and Orange Pekoe (O.P) grades of Sri Lankan black tea (mean ± SEM)

20% aqueous tea extract	SPF value
Dust No:1	35.83 ± 0.042*
B.O.P.F	22.80 ± 0.003
O.P	22.33 ± 0.004

*P<0.05

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