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Determination of sun protection factor number: an emerging *in–vitro* tool for predicting UV protection capabilities

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Abstract
The deleterious effect of ultra-violet rays from the sun on the skin is alarming and agents or substances that can reduce or combat these effects are well sought for. The purpose of this study was to investigate the potential of commonly consumed teas in providing sunscreen protection using the *in vitro* method. Thirty selected teas (green, black and "others") were brewed and diluted to produce 20% v/v aqueous solution. The absorbances of the solutions were determined by the UV spectrophotometer between wavelengths of 320 and 290 nm. The sunscreen protection factors (SPF) were then calculated using the Mansur equation. The determined SPF values were between 10.33 and 25. 33%. The green teas were observed to have the highest absorbance (2.5) and consequently higher SPF (25.33%) followed by the black teas (24.45%) and then the "others". Some of the unclassified teas however had values as high as 25.20% while also having very low values (10.33%). Teas have been postulated to have skin protective abilities when consumed orally or applied topically; this work gives further credit to this postulation. The results reveal that these safe and cheap teas have great potential as sun-protective agents.

Keywords: Herbal teas, sun protection factor, *in vitro* method

1. Introduction
The skin is the largest organ in the body, makes up to 16% of the entire body and covers about 1.8 m^2. It protects against chemicals and environmental hazards, radiation, infections; it also regulates the passage of fluids and electrolytes [1]. Excessive exposure of the skin to ultraviolet radiation generates reactive oxygen species induced by oxidative stress; this in turn triggers erythema, sunburns, skin cancer etc. The main source of ultraviolet radiation (UV) is the sun; it is the electromagnetic light/spectrum that is transmitted by the sun to the earth. Artificial sources like lamps are also capable of transmitting UV radiation [2]. There are 3 types of UV radiation namely; UVA which penetrates deep into the epidermis and dermis of the skin and generates free radicals indicated in premature skin ageing, wrinkling and spots on the skin. This type of radiation has the longest wavelength (between 400 and 320 nm) and is the major source of skin tanning whether natural or artificial. UVB radiation is the major cause of skin reddening and is responsible for the development of skin cancer because it burns the skin surface; it is a short wave radiation which reaches the earth semi-filtered in a region between 320 and 290 nm. UVC is the most dangerous of the 3, but it is prevented from reaching the earth by the ozone layer and its wavelength is between 290 and 200 nm [3, 4]. Cosmetics, herbs, teas, fruits/vegetables, oils and vitamins are implicated in the issue of SPF. These herbs, fruits, teas also have antioxidant potential, therefore, they are able to scavenge free radicals and have an effect in protecting the skin from the harmful UV radiation. The inclusion of sunscreen products is widely used globally in the cosmetic industry as this ensures adequate protection against harmful UV rays. These sunscreen products can be incorporated into oils, creams, gels and lotions. The sunscreens used in the cosmetic industry can either be physical or chemical depending on their mode of action [5]. Consequent upon the fact that it is practically impossible to be completely shielded from these rays, creams and lotions containing ingredients capable of protecting the skin have been formulated for basic everyday use. Examples of commonly used chemical ingredients used as sunscreen are zinc oxide and titanium dioxide. The naturally occurring compounds like anthocyanins, proanthocyanidin, resveratrol (grapes), quercetin, apigenin, silymarin, curcumin, vitamin E, vitamin C, wheat germ oil, pumpkin seed oil, carotenoids, herbs, teas, fruits and vegetables are also implicated in sunscreen activity [5]. These sunscreens act by absorbing, reflecting or scattering UV radiations, they also have antioxidant potentials capable of scavenging free radicals and thereby, protecting the skin from the harmful UV radiation and are now been incorporated into basic every day products like

skin and hair formulations. Teas (*Camellia sinensis*) are organic aromatic beverages prepared by infusing tea leaves in boiling water; the resultant liquid is taken as a drink, as medicine or both. They originated from the South-Western part of China and the Chinese recommend their application to skin to soothe sunburn. Teas possess strong antioxidant properties; they also have claimed medicinal properties which are due to the presence of polyphenols especially catechin. Catechin is responsible for anti-aging of the skin, prevention and repair of damaged skin and prevention of skin cancer [6]. Reports from animal studies have shown that when teas are taken orally or applied topically, they prevent or reduce adverse skin reactions that occur due to exposure to UV radiation [7]. Of particular interest is the effect of green tea when applied topically; they act as photo-protective substances that help reduce number of cells affected by UV radiation. The inclusion of tea extract in cosmetic products has been reported to show evidence of antioxidant and anti-carcinogenic properties [8].

The effectiveness of substances used as sunscreens is expressed as the protection factor i.e. the UV energy required to produce a minimal erythema on an unprotected skin within a period of time. The sunscreen protection factor (SPF) suggests the length of time you can leave your skin exposed in the sun after application of a sunscreen without getting burnt or the sun-protection ability of substances. The SPF numbers have become a worldwide standard for measuring the effectiveness of sunscreen products and these numbers can be determined by *in vitro* or *in vivo* methods. The *in vivo* method involves testing of creams/lotions containing SPF on human volunteers and this is time consuming, complex, requires ethical clearance and very expensive. The *in vitro* method on the other hand is simple, economical, practical, non-time consuming and does not require the use of life specimen. It involves determination of absorption of dilute solutions of sunscreens substances and then extrapolation of the SPF using an equation known as the Mansur equation.

$$SPF = CF\,X \sum_{290}^{320} EE\,(\lambda) X\, I\,(\lambda) X Abs\,(\lambda) \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 1$$

CF = correction factor (10), EE = erythmogenic effect of radiation with wavelength,

I = solar intensity spectrum, Abs (λ) = spectrophotometric absorbance values at wavelength.

The aim of this work was to determine the sunscreen potentials of herbal teas circulating in the Nigerian market with a view to ascertaining the veracity of the Mansur equation as an emerging popular *in vitro* tool for predicting UV protection capabilities. Literature survey shows that, this is the first time such a large number of samples would be evaluated using this *in vitro* tool.

2. Materials and Method

The twelve black teas assessed include;

Lipton tea, Top tea, Typhoo (English breakfast tea), Ketapa Pride, Alwazah tea, Hillwaytea, Richmond tea, PG tips, Al - ameer tea, Dilmah, Loyd, Impra, Ahmad

The following were the green teas assessed;

Legend green tea, Mustika Ratu, Twinings, Highland, Qualitea, 3 Ballerina

The following were neither black nor green teas and were classified as "others"; Fat burner, Beyond Comment tea, Moringa tea (Miracle), Noni tea, Moringa tea (Tra Chum Ngay), Vernonia Ocimum tea, Vidsamor Moringa tea, Healthy Hour Super Herb Sliming tea, Earl Grey tea, Darkin's Herb tea, Moringa tea with ginger.

2.1 Preparation of tea brews

Boiling water (50 mL) was poured unto one (1) g of each tea in a beaker and left to brew for 5 min. The brewed bags were squeezed through a muslin cloth and then diluted to 20% v/v using water (i.e 80 mL of distilled water was added to 20 mL of the filtrate). Absorbance and transmittance of the brewed teas were read between 290 and 320 nm in a UV spectrophotometer using a quartz cuvette at 5 nm intervals with distilled water as the blank. The readings were taken in triplicates and the mean were used to calculate the SPF numbers.

3. Results and Discussion

Many methods have been postulated for the determination of SPF spectrophotometrically such as the use of capillary film. This is inappropriate for highly viscous materials due to the inability to obtain uniform film thickness as such distorting the transmission of UV light through the film. The aqueous or hydro-alcoholic preparation of samples is the easier and widely acceptable method of spectrophotometric determination of SPF. In this study, the SPF numbers of aqueous extracts of the herbal teas were calculated by applying Mansur mathematical equation. A representative picture of the teas evaluated is shown in figure 1.

Fig 1: Photograph of the herbal teas assessed

The absorbance of the tea samples are shown in Tables I, II and III while the SPF values calculated through UV-Spectrophotometric method are shown in Table IV. The SPF values of the aqueous herbal teas range between 10.33 and 25.33. The green teas; Mustika ratu, Twinings, Qualitea and Ballerina were found to have the highest SPF values among all the teas studied. The implication is that these teas may protect the skin from harmful effects of the sun when consumed orally or applied topically. However, two of the unclassified (others); teas Earl Grey tea and Moringa tea and one of the black teas; Loyd tea were observed to have SPF below 15% indicating their relative inability for skin protection. Almost all the teas in the same category have the same or similar SPF values as calculated from Mansur equation.

Table I: Absorbance of black teas (B1 - B13)

nm	EE*I	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12	B13
290	0.0150	2.516 ± 0	2.516 ± 0	2.516 ± 0	2.492 ± 0	2.445 ± 0	2.445 ± 0	2.445 ± 0	2.445 ± 0	2.445 ± 0	2.434 ± 0	2.162 ± 0	2.434 ± 0	2.434 ± 0
295	0.0817	2.492 ± 0	2.447 ± 0	2.445 ± 0	2.445 ± 0	2.445 ± 0	2.445 ± 0	2.445 ± 0	2.445 ± 0	2.445 ± 0	2.434 ± 0	1.790 ± 0	2.434 ± 0	2.434 ± 0
300	0.2874	2.475 ± 0	2.440 ± 0	2.444 ± 0	2.444 ± 0	2.444 ± 0	2.444 ± 0	2.444 ± 0	2.444 ± 0	2.134 ± 0	2.227 ± 0	1.547 ± 0	2.205 ± 0	2.434 ± 0
305	0.3278	2.447 ± 0	2.443 ± 0	2.443 ± 0	2.443 ± 0	2.443 ± 0	2.443 ± 0	2.443 ± 0	2.443 ± 0	1.899 ± 0	1.980 ± 0	1.365 ± 0	1.960 ± 0	2.425 ± 0
310	0.1864	2.438 ± 0	2.443 ± 0	2.438 ± 0	2.438 ± 0	2.443 ± 0	2.438 ± 0	2.438 ± 0	2.438 ± 0	1.712 ± 0	1.781 ± 0	1.231 ± 0	1.774 ± 0	2.429 ± 0
315	0.0837	2.334 ± 0	2.443 ± 0	2.439 ± 0	2.439 ± 0	2.439 ± 0	2.439 ± 0	2.439 ± 0	2.439 ± 0	1.553 ± 0	1.607 ± 0	1.122 ± 0	1.605 ± 0	2.372 ± 0
320	0.0180	2.143 ± 0	2.443 ± 0	2.438 ± 0	2.443 ± 0	2.275 ± 0	2.438 ± 0	2.438 ± 0	2.440 ± 0	1.426 ± 0	1.466 ± 0	1.032 ± 0	1.468 ± 0	2.190 ± 0

Table II: Absorbance of green teas (G1 – G6)

nm	EE*I	G1	G2	G3	G4	G5	G6
290	0.0150	2.536 ± 0	2.536 ± 0	2.536 ± 0	2.536 ± 0	2.536 ± 0	2.536 ± 0
295	0.0817	2.536 ± 0	2.536 ± 0	2.536 ± 0	2.536 ± 0	2.536 ± 0	2.536 ± 0
300	0.2874	2.536 ± 0	2.536 ± 0	2.536 ± 0	2.536 ± 0	2.536 ± 0	2.536 ± 0
305	0.3278	2.528 ± 0	2.528 ± 0	2.528 ± 0	2.528 ± 0	2.528 ± 0	2.528 ± 0
310	0.1864	2.533 ± 0	2.533 ± 0	2.533 ± 0	2.266 ± 0	2.533 ± 0	2.533 ± 0
315	0.0837	2.520 ± 0	2.535 ± 0	2.535 ± 0	1.854 ± 0	2.535 ± 0	2.535 ± 0
320	0.0180	2.342 ± 0	2.535 ± 0	2.535 ± 0	1.569 ± 0	2.535 ± 0	2.535 ± 0

Table III: Absorbance of green teas (UC1 – UC11)

nm	EE*I	UC1	UC2	UC3	UC4	UC5	UC6	UC6	UC7	UC8	UC9	UC10	UC11
290	0.0150	2.520 ± 0	1.996 ± 0	2.520 ± 0	1.770 ± 0	2.528 ± 0	2.524 ± 0	2.524 ± 0	2.524 ± 0	2.281 ± 0	2.034 ± 0	2.524 ± 0	1.250 ± 0
295	0.0817	2.520 ± 0	2.520 ± 0	2.520 ± 0	2.519 ± 0	2.528 ± 0	2.524 ± 0	2.524 ± 0	2.524 ± 0	2.100 ± 0	1.716 ± 0	2.452 ± 0	1.146 ± 0
300	0.2874	2.475 ± 0	2.440 ± 0	2.444 ± 0	2.444 ± 0	2.444 ± 0	2.444 ± 0	2.444 ± 0	2.444 ± 0	2.134 ± 0	2.227 ± 0	1.547 ± 0	2.205 ± 0
305	0.3278	2.447 ± 0	2.443 ± 0	2.443 ± 0	2.443 ± 0	2.443 ± 0	2.443 ± 0	2.443 ± 0	2.443 ± 0	1.899 ± 0	1.980 ± 0	1.365 ± 0	1.960 ± 0
310	0.1864	2.438 ± 0	2.443 ± 0	2.438 ± 0	2.438 ± 0	2.443 ± 0	2.438 ± 0	2.438 ± 0	2.438 ± 0	1.712 ± 0	1.781 ± 0	1.231 ± 0	1.774 ± 0
315	0.0837	2.334 ± 0	2.443 ± 0	2.439 ± 0	2.439 ± 0	2.439 ± 0	2.439 ± 0	2.439 ± 0	2.439 ± 0	1.553 ± 0	1.607 ± 0	1.122 ± 0	1.605 ± 0
320	0.0180	2.143 ± 0	2.443 ± 0	2.438 ± 0	2.443 ± 0	2.275 ± 0	2.438 ± 0	2.438 ± 0	2.440 ± 0	1.426 ± 0	1.466 ± 0	1.032 ± 0	1.468 ± 0

Table IV: Sun Protection Factor numbers of the Teas

Black teas	SPF	Green Teas	SPF	"Others"	SPF
B1	24.42 ± 3.12	G1	25.28 ± 3.21	UC1	25.20 ± 3.19
B2	24.45 ± 3.09	G2	25.33 ± 3.20	UC2	25.12 ±3.20
B3	24.43 ± 3.09	G3	25.33 ± 3.20	UC3	25.14 ± 3.20
B4	24.43 ± 3.09	G4	24.08 ± 3.26	UC4	24.11 ± 3.14
B5	24.40 ± 3.10	G5	25.33 ± 3.20	UC5	25.17 ± 3.22
B6	24.42 ± 3.09	G6	25.33 ± 3.20	UC6	25.18 ± 3.18
B7	24.42 ± 3.09			UC7	25.18 ± 3.18
B8	24.42 ± 3.09			UC8	19.55 ± 2.47
B9	19.47 ± 2.52			UC9	14.82 ± 1.85
B10	20.17 ± 2.64			UC10	23.39 ± 2.96
B11	14.13 ± 1.81			UC11	10.33 ± 1.32
B12	20.03 ± 2.61				
B13	24.20 ± 3.08				

Previous reports have expounded the potential of natural and safe materials as sun-protective agents; for example, Patel *et al.* [9] reported the incorporation of *Pongamia pinnata* leaf and *Punica granatum* peel extracts in sunscreen formulations with antimicrobial properties, the SPF value was found to be 5 and transmittance of less than 2%. The authors concluded that, the two extracts which have been used in traditional medicine for treatment of skin diseases like eczema and leprosy could also be harnessed for use as natural sunscreen agents. In a similar but different study, Kaur and Saraf [3] investigated the potential of herbal oils in sun-protection. The duo reported SPF values ranging between 0.25 and 7.5 with olive oil having the highest value and suggested that, the information was critical in the selection of oils that could be incorporated into sunscreen formulations. Aqueous solutions of citrus juices have also been investigated for sun-protective potential due to the presence of antioxidant and found to have SPF values of about 2 which could be incorporated as ingredients in sunscreen products [10]. In another report and probably the widest in scope hitherto, the SPF of aqueous fruit extracts of five plants namely; cucumber, coconut, watermelon, strawberry, carrot, aloe vera and papaya, Malsawmtluangi *et al.* [11] observed that coconut fruit extract had the highest SPF (7.38 ± 0.22) while watermelon had very low SPF (0.97 ± 0.41). The authors equally concluded that, incorporation of natural fruits into creams or their use alone as sunscreen products would be beneficial. On their part, Ratnasooriya *et al.* [12] reported the highest SPF values for their samples which incidentally were black teas. The authors observed that Sri Lankan black tea which has high antioxidant potential also possesses SPF greater than 15 indicating their potential for good sunscreen activity. Although our observation on the behavior of different teas herein investigated are not significantly different from previous reports by other researchers, our study is larger in scope than all previous studies and unlike previous researchers, except Ratnasooriya *et al.* [13] who reported on two black teas, our study focused on all teas already approved by the regulatory agency (National Agency for Food and Drug Administration and Control; NAFDAC) and circulating in the Nigerian market. It is significant to note here that, most of these teas are imported and command high patronage. According to Guyer *et al.* [13]

"even though several synthetic sunscreens are available, they have limited applications in cosmetics due to their potential toxicity in humans and ability to interfere only in selected pathways of carcinogenesis.

Botanical and herbal agents are known to be safe and have been widely accepted by consumers. They also work in various ways by stimulating immune response, inducing gene suppression, detoxifying carcinogens, blocking oxidative damage to DNA, initiating selected pathways or by other mechanisms [5, 14]. Thus, these herbal teas play significant and multiple roles in ameliorating the process of carcinogenesis. Therefore, these herbal teas at optimum concentrations could produce several beneficial effects to the skin apart from functioning as UV filters. It is important to remember too that, teas are not only commonly consumed as drinks at home, but are also used as remedy for sunburns by applying cooled teas on the skin; the presence of theobromine and tannic acid in these teas help to remove heat from sunburns. Teas generally contain water extractable polyphenolic compounds which possess antioxidant, anti-inflammatory and anti-carcinogenic properties, these compounds destroy free radicals implicated in skin damage and thus, repair the skin. One of such polyphenolic compounds; epigallocatechin gallate which is present in green tea is said to be about 100% more potent than vitamin C as an antioxidant and 25% more effective than vitamin E as a sun-protection agent. Epigallocatechin gallate is thought to be responsible for the sun-protection ability of teas [5, 14]. The presence of these polyphenolic compounds in the different types of teas differs and as such determines their effectiveness in skin protection. The topical and even oral use of these teas has proved to be effective for photo-protection of the skin. Consumption of green teas has been reported to protect the erythrocytes membrane in the skin cells leading to reduction in sunburn reactions and its application on the skin reduced DNA damage caused by UV radiation in mice although the benefits when applied were significantly greater than when taken orally [5, 14]. Black teas have also been reported [15] to reduce skin redness after exposure to UVB radiation and they also repair the skin because of the presence of anti-oxidants. The green teas were observed to have the highest SPF (25%) followed by some of the uncategorized teas and then black teas (24%). Values between 15 and 30% have been suggested as good, as it implies that these agents can block 93-97% UVB radiation while also offering protection against UVA radiation, whereas values > 50% are capable of blocking about 98% UVB, but they offer minimal protection against UVA, which is responsible for accelerated skin aging.

4. Conclusion

The SPF values of the aqueous extracts of some commonly found teas were evaluated. It was found that most of them have the UV protection capabilities. Along with their many beneficial effects and safety, these botanicals could become good, cheap and easily available formulation ingredients in sunscreen products.

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