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Photochemoprotective Activity of Alcoholic Extract of *Camellia sinensis*

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Abstract: The main antioxidant polyphenols present in *Camellia sinensis* (green tea) are known as epicatechins which function as anti-inflammatory or anticarcinogenic agents. There are various evidences in the literature for the protective effects that green tea polyphenols exert on the skin's immune system on oral and topical application. The aim of this study was to produce green tea extract and to evaluate its photochemoprotective ability. The alcoholic extract of green tea leaves was prepared by continuous hot extraction method using Soxhlet apparatus. The antioxidant activity of the extract was assessed by reducing power estimation method in which the antioxidant activity of extract was compared with that of standard (ascorbic acid). The individual antioxidant activity obtained was 51.12±1.8% and combined antioxidant activity (ascorbic acid with extract) showed additive synergistic effect as compared to that of standard. *In vitro* SPF was determined according to the spectrophotometric method where alcoholic dilution of green tea extract was prepared and *in vitro* photoprotective activity was studied by UV spectrophotometeric method in the range of 290-320 nm. The extract produced high absorbances at 290-320 nm wavelength range and SPF obtained was 18.10±0.05 which collectively confirmed the photoprotective activity of green tea polyphenols. The higher antioxidant activity and sun protection factor of green tea extract could be utilized in the preparation of photoprotective cosmetic formulations which could prevent the skin with harmful effects of ultra violet radiations.

Key words: Epicatechins, sun protection factor, antioxidant, spectrophotometric method, polyphenols

INTRODUCTION

Photochemoprevention is the use of agents capable of ameliorating the adverse effects of ultra violet rays on skin. It has become an important tool for the fight against skin cancer (Chanchal and Swarnlata, 2009). Extracts of many plants, citrus fruits and leafy vegetables as source of ascorbic acid, vitamin E and phenolics compounds and enzymes possess the ability to reduce the oxidative damage (Ashawat *et al*., 2007a; Atrooz, 2009).

These oxidative damages ultimately reduce the protective enzyme level and imbalances the level of total protein and ascorbic acid and other antioxidants level in cells. The creams comprises such extracts could be utilized for the protection of photo induced intrinsic oxidative stress as well as structural alteration in skin (Ashawat *et al*., 2007b). Naturally occurring herbal compounds such as phenolic acids, flavonoids and high molecular weight polyphenols are very useful for prevention of the adverse effects of UV radiation on the skin (Svobova *et al*., 2003).

Green tea obtained from plant *Camellia sinensis* of the Theaceae family is one of the most widely consumed beverages in the world. The four major catechins are (-)-epigallocatechin-3-gallate (EGCG), that represents approximately 59% of the total of catechins; (-)-epigallocatechin (EGC) (19% approximately); (-)-epicatechin-3-gallate (ECG) (13.6% approximately) and (-)-epicatechin (EC) (6.4% approximately) (McKay and Blumberg, 2002). Green tea also contains gallic acid and other phenolic acids such as chlorogenic acid and caffeic acid and flavonols such as kaempferol, myricetin and quercetin (USDA, 2003).

All these polyphenols act as potent antioxidants and can scavenge ROS (reactive oxygen species), such as lipid free radicals, superoxide radicals, hydroxyl radicals, hydrogen peroxide and singlet oxygen. Afaq and Mukhtar (2006) have reported in their publications that topical application or oral feeding of a polyphenolic fraction prepared from green tea prevents photocarcinogenesis.

Numerous studies have shown that green tea extract derived from the dried fresh leaves of the plant *Camellia sinensis* and one of its major constituents, EGCG, possess obvious antiproliferative (Nihal *et al*., 2005), antiangiogenic (Tang *et al*., 2003) antimetastatic (Jung and Ellis, 2001), proapoptotic (Qanungo *et al*., 2005) and cell cycle perturbation (Ahmad *et al*., 2000) activities in various *in vitro* and *in vivo* tumor models. These experimental studies together with several epidemiologic studies have suggested that green tea extract and EGCG

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harbor strong anticancer and cancer preventive effects in numerous human cancers (Sato, 1999). The aim of this study was to produce green tea extract and to determine its photochemoprotective ability by evaluating antioxidant effect and *in vitro* sun protection factor. The green tea extract could also be incorporated into the cream formulations which could be used as the preventives from photoaging as used by Ashawat *et al.* (2007c) with few other herbal antioxidant extracts.

MATERIALS AND METHODS

The dried leaves of the plant *Camellia sinensis* were purchased from local authentic herbal distributor and were authentified with the help of herbarium of the Pharmacognosy department of University Institute of Pharmacy, Pt. Ravishankar Shukla University, Raipur. Ethanol, trichloroacetic acid, potassium ferricyamide, L-ascorbic acid and ferric chloride all were of analytical grade. Double distilled water was used for all experiments. Microcentrifuge (RM-12 C DX, Remi) and UV spectrophotometer (UV-Visible spectrophotometer 1700-Pharmaspec, Shimadzu, Japan) were used for the present study.

Preparation of extract (WHO, 2004; Rajpal, 2004): Extraction of the green tea leaves was done by hot extraction process using Soxhlet apparatus. The 350 g of weighed green tea leaves were taken, made to coarse powder and packed in the Soxhlet apparatus. The defatting was done by petroleum ether at 60-80°C. Then extraction was performed with ethyl alcohol (90%) at temperature 50-60°C. The extract was concentrated by recovering the solvent and then dried the extract and weighed to get actual percent yield of the extract. It was calculated from the obtained mass and initial powdered drug.

Antioxidant activity determination: The antioxidant activity of the extract was assessed by reducing power estimation method in which the antioxidant activity of extract was compared with that of standard (ascorbic acid).

The relative reducing activity in terms of antioxidant activity of green tea extract was determined by using 5 mg of extract as well as its combination with equal amount of ascorbic acid. The extract (5 mg) and ascorbic acid (5 mg) were dissolved separately in 1.0 mL of deionized water with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and 1% potassium ferricyamide (2.5 mL). The mixture was incubated at 50°C for 20 min. Aliquotes of trichloroacetic acid (2.5 mL, 10% w/v) were added to the mixtures and centrifuged at 3000 rpm for 10 min. The upper layer of solution (2.5 mL) was mixed with distilled water (2.5 mL) and a freshly prepared ferric chloride solution (0.5 mL, 0.1%). The complete mixture was diluted upto 10 mL with distilled water and further took 1 mL and diluted upto 10 mL with distilled water. The absorbance was measured at 700 nm by UV-visible spectrophotometer (1700-Pharmaspec, Shimadzu, Japan) by taking as blank the mixture of all the reagents in the same quantity as added while preparing samples. Increased absorbance of the reaction mixture indicated increased antioxidant activity via reducing power with reference to equal amount of standard ascorbic acid (Rajeshwar *et al.*, 2005; Ashawat *et al.*, 2007b). Similar procedure was repeated to know combination antioxidant power of extract with ascorbic acid as shown in Table 1.

Sun Protection Factor (SPF) determination: The efficacy of a sunscreen is usually expressed by the Sun Protection Factor (SPF) which is defined as the UV energy required to produce a Minimal Erythema Dose (MED) on protected skin, divided by the UV energy required to produce a MED on unprotected skin:

$$SPF = \frac{\text{Minimal erthema dose in sunscreen} - \text{Protected skin}}{\text{Minimal erythema dose in non sunscreen} - \text{Protected skin}}$$

The Minimal Erythemal Dose (MED) is defined as the lowest time interval or dosage of UV light irradiation sufficient to produce a minimal, perceptible erythema on unprotected skin (Aburjai and Natsheh, 2003; Bendova *et al.*, 2007). The higher the SPF, the more effective is the product in preventing sunburn. *In vitro* screening methods may represent a fast and reasonable tool reducing the number of *in vivo* experiments and risks related to UV exposure of human subjects, when the technical test parameters are adjusted and optimized (Wood and Murphy, 2000).

The *in vitro* SPFs were determined according to the method described by Mansur *et al.* (1986), Santos *et al.* (1999), Ashawat *et al.* (2006) and Kaur and Saraf (2010).

Table 1: Antioxidant activity of ethanolic extracts

Aliquots	Absorbance at 700 nm	Antioxidant activity (%)
Ascorbic acid	1.066±0.00***	100.00
C. sinensis extract	0.545±0.00***	51.12±1.80
Ascorbic acid and *C. sinensis* extract in combination	1.689±0.00***	158.44±0.20

Values are Mean±SD for n = 3, ***p<0.001

Table 2: SPF determination of green tea extract

Wave length (nm)	EE (λ)×I (λ) employed	Absorbance (A)	EE(λ)×I (λ)×Absorbance (A)
290	0.0150	1.930±0.001	0.028965±0.000015
295	0.0817	1.925±0.001	0.1573542±0.000081
300	0.2874	1.893±0.002	0.544623±0.000574
305	0.3278	1.828±0.011	0.6028242±0.003605
310	0.1864	1.726±0.001	0.3219128±0.000186
315	0.0837	1.600±0.001	0.1340037±0.000083
320	0.0180	1.397±0.002	0.025182±0.000036

Σ EE (λ)×I (λ)×A = 1.810±0.005, SPF = Σ EE(λ)×I (λ)×A×10 (Correction Factor), SPF = 1.8101×10 = 18.10±0.05

For sample preparation 100 mg of green tea extract was weighed and made up the volume upto 10 mL with ethanol which gave 10,000 micro g mL^{-1} of extract. Then 1 mL was taken out of it and made up the volume upto 10 mL which gave 1000 μg mL^{-1}. Further took 2 mL of the above dilution and made up the volume upto 10 mL which produced 200 μg mL^{-1} of the extract. Then absorbance values of each aliquot prepared were determined from 290-320 nm, at 5 nm intervals, taking ethanol as blank for extract aliquots and distilled water for cream aliquots, using Shimadzu UV-Visible spectrophotometer (Shimadzu 1700, Japan) values are shown in Table 2.

The observed absorbance values at 5 nm intervals (290-320 nm) were calculated by using formula:

$$SPF_{spectrophoolometric} = CF \times \sum_{290}^{230} EE(\lambda) \times 1(\lambda) \times Abs(\lambda)$$

where, CF is correction Factor (10), EE (λ) is erythmogenic effect of radiation with wavelength λ and and Abs (λ) is spectrophotometric absorbance values at wavelength λ. The values of EE (λ)×I (λ) are constants. The obtained absorbance values Abs (λ) were multiplied with the respective EE (λ)×I (λ) values as given by Sayre *et al.* (1979). Then their summation was taken and multiplied with the correction factor (10) as depicted in Table 2.

Statistical analysis: Statistical analysis was carried out using STAT software14, the measurements were taken thrice and the values were expressed in Mean±Standard deviation. All parameters were statistically analyzed at 95% confidence level. One-way ANOVA analysis, with Tukey correction was used to determine significant differences for the comparison. Differences were considered statistically significant if $p<0.001$.

RESULTS AND DISCUSSION

On taking initially 350 g of green tea leaves coarse powder, after complete extraction 63.875 of concentrated green tea alcoholic extract was obtained. Thus practical yield obtained was 18.25% w/w which was appreciably good amount. The antioxidant activity of the extract was assessed by reducing power estimation method in which the antioxidant activity of extract was compared with that of standard (ascorbic acid). As observed in the Table 1 the absorbance value for ascorbic acid was considered 100% antioxidant activity and when we compared absorbance of green tea extract as compared to ascorbic acid it produced 51.12±1.8%. Similarly when equal amount of ascorbic acis and green tea extract was taken the antioxidant activity came to be 158.44±0.20,which clearly shows the additive synergistic effect with ascorbic acid. As work done by Ashawat *et al.* (2007a) on *A. catechu* and *P. granatum* and Adesegun *et al.* (2008) on *Sapium ellipticum*. It was observed that antioxidant activity was produced due to the presence of phenolic compounds. Similarly it could be concluded that as *C. sinensis* containes polyphenolic flavonoids named as epicatechins which can scavenge ROS (reactive oxygen species, such as lipid free radicals, superoxide radicals, hydroxyl radicals, hydrogen peroxide and singlet oxygen). Afaq and Mukhtar (2006) have reported in their publications that topical application or oral feeding of a polyphenolic fraction prepared from green tea prevents photocarcinogenesis. The assessment of antioxidant activity proves the photoprotective nature of green tea extract.

Antioxidant properties of medicinal plants have been shown to be due to high content of phenolic compounds (Vinson *et al.*, 2001; Meyers *et al.*, 2003). Polyphenols have a high redox potential which allows them to act as reducing agents, hydrogen donors and singlet oxygen quenchers (Kahkonen *et al.*, 1999). The antioxidant effects of the extract may be due to its phenolic content. In the reducing power assay, the antioxidants in the extract would cause the conversion of Fe^{3}+/ferricyanide complex to the ferrous form. The reducing power increased with increasing concentration of the extract and the standards. This shows that the reducing ability of green tea extract was evident. This suggests that the extract is an electron donor and could neutralize free radical (Zhu *et al.*, 2001).

Second parameter taken was *in vitro* SPF determination. The SPF is a quantitative measurement of the effectiveness of a sunscreen formulation. To be effective in preventing sunburn and other skin damage, a

sunscreen product should have a wide range of absorbance between 290 and 400 nm. The *in vitro* SPF is useful for screening test during product development, as a supplement of the *in vivo* SPF measure. In this research alcoholic green tea extract was evaluated by UV spectrophotometry applying Mansur mathematical equation. SPF values of samples obtained using the UV spectrophotometric method are shown in Table 2. We can clearly see that high absorbance values were obtained at 290-320 nm wavelength range. The value of SPF obtained was 18.10±0.05 which is very appreciating when we compare with that of other herbal extracts as reported by various authors. For e.g., SPF of *Boerhavia diffusa* was between 3.539±0.0213 to 7.1747±0.0038 (Ashawat *et al*., 2006) and of fresh Aloe vera gel was 0.995±0.221 (Ashawat *et al*., 2008). In contrast, Khazaeli and Mehrabani (2008) determined *in vitro* SPF values of extracts of sixteen medicinal plants and reported that extracts of leaves of *Dracocephalum moldavica* L. and flowering tops of *Viola tricolor* L. had high SPFs i.e., 24.79 and 25.69, respectively and concluded that these plants had high amounts of phenolic compounds and flavonoids which could be the cause for their high SPF.

It has been reported that topical application of green tea extract prior to UV B radiation result in significant protection against UVB induced cutaneous edema and erythema. Hence green tea extract could form an important constituent of photoprotective formulations. By adding various constituents producing synergistic effects with herbal extract and its photoprotective activity high range herbal photoprotective formulations could be designed.

CONCLUSION

By the antioxidant activity determination it was found that *Camellia sinensis* extract is an effective antioxidant and it also produced synergistic effect with ascorbic acid. Thus it could be used in herbal topical photoprotective formulations. Thus natural antioxidants alone or in combination with synthetic chemical antioxidants may produce multiple effects on skin. The proposed UV spectrophotometric method is simple, rapid, employs low cost reagents and can be used in the *in vitro* determination of SPF values in many cosmetic formulations. The proposed methodology may be useful as a rapid quality control method. It can be used during the production process in the analysis of the final product and can give important information before proceeding to the *in vivo* tests. Similarly the high value of SPF obtained also supports to the photoprotective action of green tea extract. Thereby, we can say that natural photoprotectives could be successfully used as they will produce effect and reduce the side effects produced by the long term effects of chemical antioxidants.

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