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Evaluation of Antioxidant Activity of ANTI SPOT (in vitro)

The antioxidant activity of the cream was evaluated in comparison to that of ascorbic acid. Their ability to scavenge the stable 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical as noted by the discoloration of DPPH solution in methanol after addition of the antioxidant was assessed (Brand-Williams, Kim Blois MS.).

Aliquots of each sample (cream or ascorbic acid solution) were diluted with the appropriate amount of methanol to a final volume of 50µl. 2,95ml of 1mM DPPH solution in 80% (v/v) aqueous methanol were added to each sample and incubated for 30 min at RT. The DPPH solution was freshly prepared and kept in the dark at 4°C. The absorbance of each sample was measured spectrophotometrically at 517 nm. Blank samples contained the same amount of sample. Control sample contained the same amount of methanol and DPPH. All measurements were performed in triplicate. The radical scavenging activity of the samples expressed as percentage of inhibition (I%) were calculated according to the following equation (Yen and Duh, 1994, Floegel et al. 2011).

$$I\% = 100 - \frac{[Abs\ (sample) - Abs\ (blank)] * 100}{Abs\ (control)}$$

Where [Abs (sample)- Abs (blank)] is the absorbance of sample using as blank the solution of sample in 80% (v/v) aqueous methanol. Abs (control) is the absorbance values of the control sample. The concentration of sample required for 50% inhibition was determined and represented as IC50 value. A percent inhibition versus concentration curve was plotted for ascorbic acid and the concentration required for IC50 was calculated.

Results summary

The concentration of the sample or ascorbic acid solution required for 50% inhibition of the free radical (IC50) is summarized on Table 1.

Table1: IC50 for Anti Spot and Ascorbic acid solution	
Sample	Concentration (μg/mL)
ANTI SPOT	258.33
Ascorbic acid	3.52

Conclusion:

ANTI SPOT caused inhibition of the free radical DPPH. Ascorbic acid, a known antioxidant was used as control.

The initial concentration of the sample was 258.33μg/ml. taking into account the fact that the antioxidant ingredient in the cream was 1.0% its concentration in the sample is calculated 2.58μg/ml.

The results indicate that the antioxidant activity of a 258.33μg/ml cream solution (antioxidant ingredient in sample: 2.58μg/ml) is equivalent with that of a 3.52μg/ml solution of ascorbic acid.

According to this result it may be concluded that ANTI SPOT has a very good antioxidant capacity.

Literature

1. Brand-Williams, W, Cuvelier, M E, Berset C: Use of a free radical method to evaluate antioxidant activity (1995) *Lebensm.-Wiss. Technol.*, **28**, 25-30
2. Kim D-O, Lee, KW, Lee HJ, Lee CY: Vitamin C equivalent antioxidant capacity (VCEAC) of phenolic phytochemicals (2002) *Journal of Agricultural and Food Chemistry*, **50**, 3713–3717