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Original Article

Evaluation of the antioxidant efficacy of extracts/ ingredients used in skin care products

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Abstract
The so-called active ingredients in skin care product formulations are purported to deliver the intended functions of the product. Active ingredients, such as the antioxidants can efficiently protect the skin if the activities are retained after incorporating into the base matrices in the product formulation. Here, we investigated the antioxidant activities of 24 extracts/compounds that are being used in skin care formulations and their ability to retain the activities (efficacy) after being mixed with the base matrices. The antioxidant activities were evaluated using 2, 2′-azino-bis (3- ethylbenzothiazoline-6-sulphonic acid (ABTS) and diphenyl-picryl hydrazine (DPPH) assays. To test the efficacy of the samples in formulation, the samples were mixed with base cream 1%, 2% and 4% (w:w) and allowed to settle overnight. Results were expressed as either percentage inhibition (%) or IC50 (µg/mL). Out of the 24 samples, ten exhibited significantly high antioxidant activities with resveratrol> pomegranate> green tea> mango> amla> bearberry> ellagic acid> tetrahydrocurcuminoid> *Rhodiola rosea*> kakadu plum in the ABTS assay. In the DPPH assay, green tea> pomegranate> tetrahydrocurcuminoid> mango> amla> resveratrol> bearberry> *Rhodiola rosea*>kakudu plum>ellagic acid. Four out of the ten samples (amla, green tea, mango and pomegranate extracts) had IC50 value lower than Trolox standard and were included in the efficacy test. Trolox standard and amla extract seemed to retain their antioxidant activities in the formulations, while green tea and pomegranate extracts had a decrease in activities. Only mango extract had a synergistic effect with the cream base with higher antioxidant activity observed compared with the extract alone. This study demonstrates the potential interaction between active and vehicle compounds, which may hinder or enhance the activities of the active ingredients in the final product. The outcome of the research has an impact in the cosmetic product formulation particularly in the quality control, chemistry and efficacy of the finished products.
Keywords: Antioxidant, Cosmetic, Active ingredients, ABTS, DPPH, Mango extract

Introduction

The human skin is continuously exposed to various environmental insults (cigarette smoke, pollutants, infrared radiation, ultraviolet radiation etc) that are damaging to the skin, leading to skin aging. The characteristics of aged skin such as wrinkles, pigmentation, sagging, dryness and dullness are aesthetically undesirable.[1,2] Many strategies and therapies for skin aging have been developed and topical application of cosmetic products with claimed benefits (anti-aging, anti-wrinkle etc.) has been one of the most popular interventions by consumers as they are perceived to be cost effective, less invasive and with minimal risk of side effects.[3]

Cosmetic products with claimed benefits are commonly termed as "cosmeceuticals", a word coined by merging the word cosme-(tic) and (pharma)-ceutical, to describe "active" and science based cosmetic.[4,5] The actives in the product formulation are expected to deliver their functions especially after mixing with other ingredients in the product formulation. Some of the commonly used actives are antioxidants.[6–9] The use of antioxidants in product formulation is due to understanding the role of oxidative stress in the aging of the skin.[10–14] In this study, we evaluated the antioxidant activities of 24 extracts/ compounds used in skin care formulations and their ability to retain their efficacy in product formulation.

Methods

Samples

All the 24 commercial extracts and the cream base were

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supplied by Hexis Lab Limited, UK.

Sample Preparation

Samples were dissolved in either water or ethanol at 4000 µg/mL. The samples dissolved in water were willow bark extract, green tea extract with EGCG, fucoidan, pomegranate extract, pullulan botanical extract, amla extract, *Rhodiola rosea* extract, mango extract and galangal extract. The samples dissolved in ethanol were bearberry extract, kakadu plum extract, tetrahydrocurcuminoid, sea buckthorn oil, resveratrol, *Moringa oleifera* extract, *Centella asiatica* whole extract and *Centella asiatica* with 2 % asiaticoside. The samples dissolved in ethanol were bearberry extract, kakadu plum extract, tetrahydrocurcuminoid, sea buckthorn oil, resveratrol, *Moringa oleifera* extract, *Centella asiatica* whole extract, *Centella asiatica* with 2 % asiaticoside, oleuropein 40%, nobiletin, aloe extract and L-carnosine.

Antioxidant Assays

A working solution of 1000 µg/mL diluted from the stock was used in the screening assay. The antioxidant assays were followed from previous methods with some modifications as described below.[15]

2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid (ABTS) Assay

ABTS radical was pre-formed from the overnight 1:1 chemical reaction of 15 mM ABTS and 5 mM potassium persulfate. The ABTS stock was diluted 1:50 with 5 mM PBS, pH 7.4 to obtain 50 mL ABTS working solution. In the assay, either Trolox standard solutions (10 µL), plant samples (10 µL) or 70% ethanol (10 µL) for controls were thoroughly mixed with ABTS working solution (290 µL) in assay wells. The microplate was subsequently incubated in the dark at 37°C for 6 minutes. All experiments were performed in triplicate. The absorbance was determined using a microplate reader, SpectraMax Plus^{384}, Molecular Device Corporation, at 734 nm. Background absorbance was corrected by subtracting the absorbance value of blank (water/ 70% ethanol).

Diphenyl-Picryl Hydrazine (DPPH) Assay

DPPH was diluted in 100% methanol at 0.24 mg/mL and allowed to settle overnight at 4°C. The stock was diluted at 1:5 with 50% methanol to obtain the DPPH working solution.15 µL each of Trolox standards, plant samples and solvents (water or 70% ethanol) as control wells were mixed with 285 µL of DPPH working solution in the assay wells. The 96 wells plate was incubated in the dark at 30 °C for 30 minutes. After incubation, the microplate was read at the wavelength of 517 nm using a microplate reader SpectraMax Plus^{384} (Molecular Device Corporation). Background absorbance was corrected by subtracting the absorbance value of blank (water/ 70% ethanol).

The ABTS and DPPH percentage inhibition were calculated as:

Percentage inhibition (%) = (1-(S/C)) x100;

S=corrected absorbance value for sample and C=corrected absorbance value for control

IC_{50} Determination

Samples with high antioxidant activities were prepared at lower concentrations to evaluate IC_{50} value. The IC_{50} value was determined from the best fitted line for each sample ($R^2 \approx 1.00$).

Efficacy Test

Four of the 24 tested samples were selected for the efficacy test. The selection was based on the samples with IC_{50} lower than Trolox standard in both assays to ensure the antioxidant activities exhibited were independent of the free radicals introduced. The selected actives were mixed with the base cream at 1%, 2% and 4% active: cream (w:w). The mixtures were allowed to settle overnight at 4°C. ABTS and DPHH assays were performed as described above. The cream base supplied consisted of mixtures of aqua, *Helianthus annuus* oil, cetyl alcohol, glycerin, *Cocos nucifera* oil, PEG-100 stearate, cetearyl alcohol, *Prunus amygdalus* oil, *Simmondsia chinensis*, *Theobroma cacao* butter, *Chondrus crispus*, benzyl alcohol, phenoxyethanol, potassium sorbate and tocopherol. The final concentration of the active at 1, 2 and 4% formulation in the ABTS assay were 1.3 µg/mL, 2.6 µg/mL and 5.2 µg/mL. The final assay concentration of the active at similar percentage formulations in the DPPH assay were 2 µg/mL, 4 µg/mL and 8 µg/mL. For comparison, sample alone at similar concentration for each formulation was investigated.

Statistical Analysis

ANOVA was used to compare the mean differences between samples and $P < 0.05$ was considered as significant.

Results

Screening

Significantly high antioxidant activities were observed with the following extracts/ compounds; amla extract, bearberry extract, ellagic acid, green tea with 90% EGCG, kakadu plum extract, mango extract, pomegranate extract, resveratrol, *Rhodiola rosea* extract, tetrahydrocurcuminoid and oleuropein 40%), whereas low to intermediate antioxidant activities were observed for: *Centella asiatica* whole extract, *Centella asiatica* with 2% asiaticoside and *Moringa oleifera* leaf extract' and low to no activity for fucoidan, nobiletin, galangal extract, kojic acid dipalmitate, niacinamide, pullulan, sea buckthorn oil, aloe extract, L-carnosine and willow bark extract (Table 1).

Comparison of IC_{50} (ABTS and DPPH Assay)

Ten samples with 80-90 % radical's inhibitions for both assays (amla extract, bearberry extract, ellagic acid, green

Table 1. Antioxidant Activities of Actives

Samples	ABTS Percentage Inhibition (%)	DPPH Percentage Inhibition (%)
High Activity		
1. Amla extract	99.1 ± 0.07	93.9 ± 0.20
2. Bearberry extract	99.6 ± 1.30	91.2 ± 0.35
3. Ellagic acid	98.6 ± 0.08	91.0 ± 0.34
4. Green tea with 90% EGCG	99.6 ± 0.03	93.2 ± 0.40
5. Kakadu plum extract	95.7 ± 4.50	90.6 ± 1.80
6. Mango extract	98.1 ± 1.16	88.9 ± 0.21
7. Pomegranate extract	99.1 ± 0.07	93.3 ± 0.08
8. Resveratrol	98.5 ± 0.76	90.3 ± 0.87
9. *Rhodiola rosea* extract	86.9 ± 1.48	89.4 ± 0.15
10. Tetrahydrocurcuminoid	98.7 ± 0.44	90.8 ± 1.14
11. Oleuropein 40%	99.1 ± 0.04	53.4 ±1.67
Low-Intermediate Activity		
12. Centella asiatica whole extract	12.0 ± 0.91	10.5 ± 0.20
13. Centella asiatica with 2% asiaticoside	14.5 ± 0.87	5.72 ± 1.02
14. Moringa oleifera leaf extract	43.3 ± 3.22	17.2 ±4.30
Low to no Activity		
15. Fucoidan (brown seaweed)	10.2 ± 2.37	-5.00 ±1.10
16. Nobiletin	9.76 ± 0.41	-4.22 ± 0.94
17. Galangal extract	2.36 ± 4.71	7.12 ±4.85
18. Kojic acid dipalmitate	0.37 ± 1.91	-2.00 ± 1.27
19. Niacinamide	-1.61 ± 1.06	-2.77 ± 5.28
20. Pullulan	2.51 ± 2.33	-4.37 ± 2.17
21. Sea buckthorn oil	4.43 ± 8.36	-14.88 ± 2.61
22. Aloe extract	2.82 ± 2.59	0.91 ± 2.64
23. L-carnosine	4.95 ± 0.9	-2.94 ± 7.36
24. Willow bark extract	1.35 ± 1.17	-6.90 ± 4.90

The samples were grouped into high, low-intermediate and low to no activities. The samples were tested at 1000 µg/ mL with final concentrations of 3.3 and 2.0 µg/mL for ABTS and DPPH assay respectively. High = 80%-90% inhibition, low-intermediate = 10-50 % and low-no activity = below 10%. Data are mean ± SD (n = 4).

tea with 90% EGCG, kakadu plum extract, mango extract, pomegranate extract, resveratrol, *Rhodiola rosea* extract and tetrahydrocurcuminoid) were evaluated for potency. In Figure 1, samples labelled 1, 2, 3, and 4 (amla extract, green tea extract, mango extract and pomegranate extract) had higher potency, observed from lower IC_{50} than Trolox standard in both assays. The four samples were further evaluated for efficacy in cream matrices.

Efficacy Test (1%, 2% and 4% Active: Cream Formulation)

1% Formulation. Figure 2 shows significant increase in antioxidant activities for mango extract mixed with cream in both assays. However, green tea and pomegranate extract in cream had a decrease in activities in both

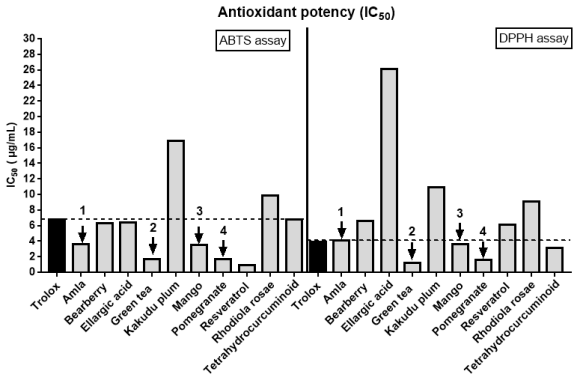

Figure 1: Comparison of IC_{50} Values of Selected Samples. Samples labelled 1, 2, 3 and 4 had lower IC_{50} than Trolox standard in both assays and were further evaluated for efficacy.

assays. Trolox and amla extract were either retained or had increase in antioxidant activities in the formulation.

2% Formulation. In a 2% formulation (Figure 3), Trolox standard retained its activity, but green tea had lower activity when mixed with cream in both assays. Amla, mango and pomegranate extract retained their activities in only one assay. Mango extract had significantly higher antioxidant activity when in cream, while amla and pomegranate had lower antioxidant activities when in cream.

4 % Formulation. In a higher percentage formulation, mango extract consistently had higher antioxidant activities in both assays (Figure 4), while Trolox and amla extracts retain their activities. Green tea with 90% EGCG and pomegranate extracts were not included at 4% formulation because the assay's limit was reached for both samples based on their IC_{50} value (Figure 1).

Discussion

Common approaches in the cosmetic product development focus on the inclusion of "actives", such as botanical extracts or pure compounds in the product formulation with intended effects to promote healthy skin and/or manage the characteristics of aged skin. Actives, such as antioxidants are believed capable of diminishing the oxidative damages caused by free radicals, potentially by restoring the balance between endogenous antioxidants and the oxidants.[7,9,16]

In this study, we demonstrated that botanical extracts possessed high antioxidant capacities as pure compounds (resveratrol and tetrahydrocurcuminoid) (Table 1). However, some of the extracts (*Centella asiatica*, *Moringa oleifera*, galangal, willow bark, aloe, fucoidan, sea buckthorn oil) had low or no activity, may be due to the extraction methods used to prepare the samples.[17–20] The other samples may not be active as antioxidants, such as kojic acid dipalmitate and nobiletin which are being used as skin whitening agents,[21,22] niacinamide as anti-bacterial and anti-inflammatory[23] and pullulan as a natural polymer

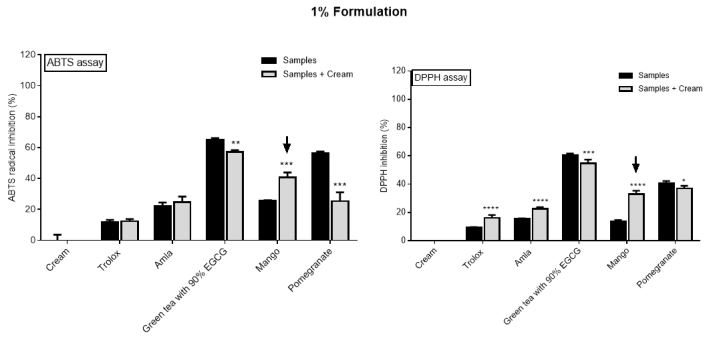

Figure 2: 1% Formulation. Comparison of antioxidant activities between sample alone *vs.* sample + cream in ABTS and DPPH assays. Only mango extract + cream showed an increase in activity in both assays compared to extract alone (Arrow). Data are the mean ± SD (n=4) with $P < 0.05$ considered as significant.

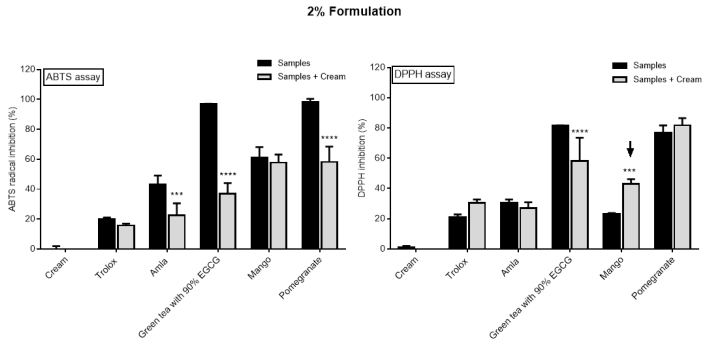

Figure 3. 2% Formulation. Comparison of antioxidant activities between sample alone *vs.* sample + cream in ABTS and DPPH assays. Mango extract + cream has higher antioxidant in the DPPH assay (arrow). Data are the mean ± SD (n=4) with p< 0.05 considered as significant.

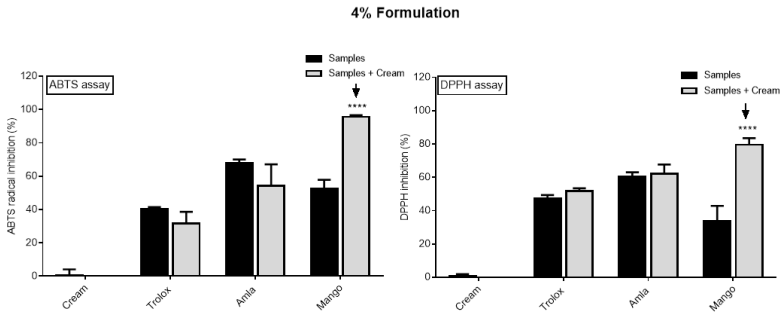

Figure 4. 4% Formulation. Comparison of antioxidant activities between sample alone *vs.* sample + cream in ABTS and DPPH assays. Data are the mean ± SD (n=4) with $P < 0.05$ considered as significant.

to enhance delivery of drugs or cosmetic agents.[24]

Comparison of sample's potency, expressed as IC_{50} (Figure 1), showed four samples (amla, green tea, mango, pomegranate) to have lower IC_{50} compared with Trolox in both assays. Of the 4 samples, we demonstrated that the mango extract in the cosmetic formulation exhibited a consistent synergistic interaction with the other ingredients in the cream base. Mango extracts has been shown to have protective effects against skin aging by being able to reduce wrinkle formation and increase collagen bundle.[25] With an enhance activity in cream formulation, the use of mango extract in this formulation will ensure the ability of the product to retain claimed effect. Although, the mechanisms involved is unclear, its active compound (mangiferin, anthocyanin and carotenoids) were postulated to exert synergism.[26,27] Similar synergistic effect, respective to the combinations, was observed in a study investigating synergistic antioxidant effects of fruits, vegetables and legumes extracts.[28] However, antagonisms effect may also occur within a formulation. Green tea and pomegranate were observed to have significant reduction at almost all percentage formulation. The

observed results suggest antagonistic effect or instability of the extracts within the formulation. For example, green tea was found to be pH-dependent, where the green tea and its component were unstable and easily degraded in alkaline solution than in acidic solution.[29,30] Evaluation the potential interactions between ingredients in a cosmetic formulation is a critical aspect to ensure product efficacy.

Conclusion

This study provided information on the antioxidant activities of commonly used extracts/compounds and the potential interaction between ingredients in a cosmetic formulation. However, the reported activities of synergism or antagonism between the studies extracts were respective to the cream ingredients.

Conflict of Interests

None.

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