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SAFETY ASSESSMENT OF OIL PALM PHENOLICS AS ACTIVE INGREDIENT FOR TOPICAL APPLICATION

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ABSTRACT

Oil palm phenolics (OPP) are water-soluble antioxidants derived from palm oil milling effluent. They contain flavonoids, polyphenols, phenolic acids, water-soluble vitamins and organic acids. As a potential new ingredient for consumer products, safety assessment is therefore very important. This article describes the safety assessment of OPP as an active ingredient for topical application. The irritation potential of OPP to the eye and skin were assessed using the in vitro *Ocular and Dermal Irritection Assay Systems respectively. For the purpose of comparison, a commercial green tea extract at the same concentrations was also evaluated. The* in vitro *ocular irritection assay study classified OPP as non-irritant or minimal irritant, but commercial green tea as mild irritant. For* in vitro *dermal studies, the results showed that both ingredients at 50% concentration and higher were classified as irritant. However at a 5% concentration, green tea was predicted as irritant, while OPP was non-irritant. Both the* in vitro *systems predicted formulated products containing 5% OPP and green tea as non-irritant. The irritation potential of the actives and formulated products with 5% actives was further evaluated via an* in vivo *patch test for 48 hr, as well as an* in vivo *human repeated patch test. It was found that both actives did not induce any irritative or allergic reactions related to the presence and activity of common allergens.*

Keywords: oil palm phenolics, green tea, skin irritation, skin sensitisation.

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INTRODUCTION

The term phenolics encompasses approximately 8000 naturally occurring compounds widely distributed in nature (Svobodova *et al.*, 2003). They are simple compounds present in most fresh fruits and vegetables, or complex compounds present in bark, roots and leaves of plants. Phenolics have one or more hydroxyl groups attached directly to an aromatic ring (*Figure 1*) (Vermerris and Nicholson, 2006).

Three important groups for human use are phenolic acids, flavonoids and high molecular weight polyphenols (Svobodova *et al.*, 2003). Naturally occurring phenolic acids are usually divided into two main groups: derivatives of benzoic acids contain-

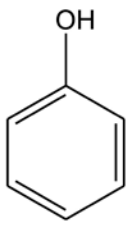

Figure 1. Structure of phenol.

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ing seven carbons such as p-hydroxybenzoic, gallic acid, ellagic acid and derivatives of cinnamic acids comprising nine carbon atoms such as p-coumaric acid, caffeic acid and ferulic acid. Flavonoids contain two or more aromatic rings, each in turn bearing one or more phenolic hydroxyl groups linked through a three carbon bridge that is usually an oxygenated heterocycle (*Figure* 2). High-molecular weight polyphenols, commonly known as tannins, are polymer compounds.

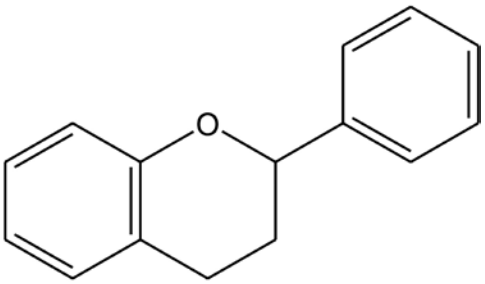
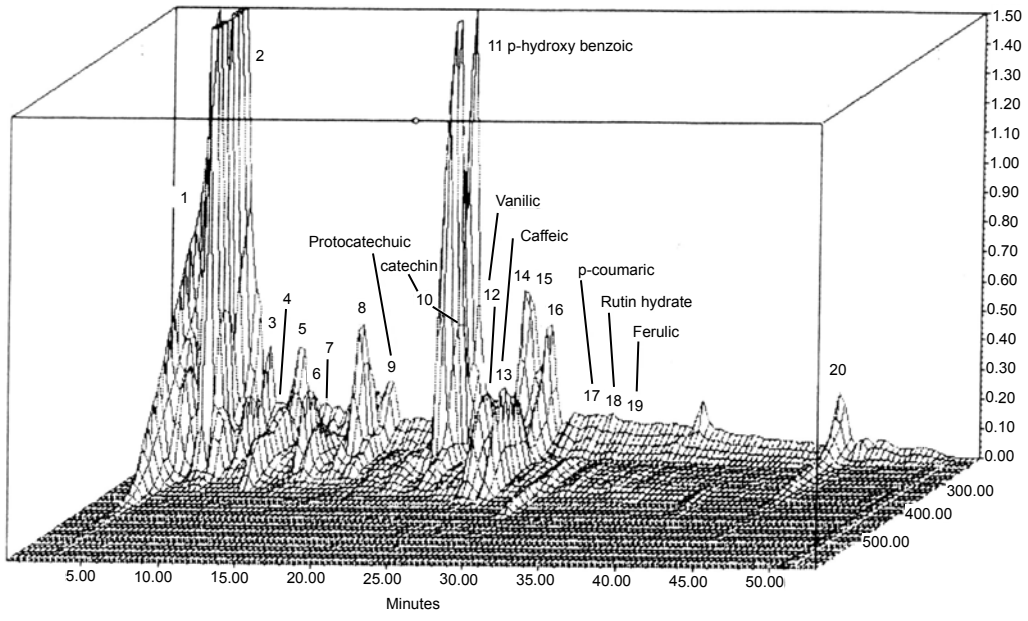


Figure 2. Basic structure of flavonoid skeleton.

The major antioxidant capacities of plants such as fruits and vegetables are provided by vitamins C and E and phenolic compounds, especially flavonoids. Phenolic compounds possess different biological activities, but the most important is the antioxidant activity. Their contribution to the antioxidant capacity of the human diet is much larger than that of vitamins. The total intake of polyphenols in a person's diet could amount to 1 g a day, whereas combined intakes of beta-carotene, vitamins C and E from food most often is around 100 mg a day (Sahelian, 2009; Scalbert *et al.*, 2005). Current evidence strongly supports the contribution of polyphenols to the prevention of cardiovascular diseases, cancers and osteoporosis. They also play a role in the prevention of neurodegenerative diseases and diabetes mellitus (Scalbert *et al.*, 2005). Studies on flavonoids such as quercetin, luteolin and catechin have shown that they are better antioxidants than the nutrients vitamin C, vitamin E and beta-carotene. Therefore, phenolics may be beneficial in preventing UV-induced oxygen free radical generation and lipid peroxidation, *i.e.* events involved in phatological states such as photoaging and skin cancer. The antioxidant properties of phenolic compounds act as free radical scavengers, hydrogen donators, metal chelators and singlet oxygen quenchers (Svobodova *et al.*, 2003).

Oil palm is one of the richest sources of fat-soluble antioxidants such as carotene, tocopherol and tocotrienol. While much attention has been focused on the fat-soluble components of palm, little emphasis was given to the water-soluble components, most of which are discarded during the milling process (Tan *et al*., 2007). A recent innovation has led to the recovery of water-soluble antioxidants from palm oil milling effluent (POME), characterised by its high content of phenolic antioxidants including flavonoids, polyphenols, phenolic acids and water-soluble vitamins, and organic acids (Sundram *et al.*, 2001; Halimoon *et al.*, 2003; Sambanthamurthi *et al*., 2005). The oil palm phenolics (OPP) account for about 2.4% of POME on a dry weight basis. *Figure 3* shows the phenolic acids of POME. Four benzoates isolated were vanillic (4.62 ppm, 0.91%),



Source: Sambanthamurthi *et al*. (2005).

Figure 3. HPLC of phenolic components in crude extract of palm oil mill effluent (POME) (Sambanthamurthi et al., 2005).

protocatechuic (7.76 ppm, 1.34%), p-hydroxybenzoic acids (127.44 ppm, 19.63%) and rutin hydrate. Three others belong to the cinnamic group, *i.e.* caffeic (6.48 ppm, 0.36%), p-coumaric and ferulic acid. Other compounds detected include fruit sugars, organic acids and water-soluble vitamins.

The major components in OPP include *p*-hydroxybenzoic acids having a general structure as shown in *Figure 4.* The methyl and propyl esters of *p*-hydroxybenzoic acid are commonly used in the food industry as anti-microbial agents and in personal care products as preservatives (Tan *et al*., 2007).

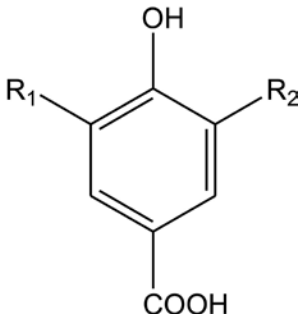


Figure 4. Chemical structure of hydroxybenzoic acids.

These palm extracts show superior free radical scavenging activity compared to that of green tea (Sundram *et al*., 2001) on an equivalent weight basis. Studies by Balasundram *et al.* found that the total phenolic content of crude and ethanolic extracts were 40.3±0.5 and 49.6±0.6 mg gallic acid equivalent (GAE) g^{-1} extract (dry basis) respectively and the radical scavenging activity indicated that both crude and ethanol extracts exhibited hydrogen-donating capacity and had anti-radical power (ARP) comparable to ascorbic acid (Balasundram *et al*., 2003; 2005). Further antioxidants activity studies on OPP in comparison to tea, commercial catechin and gallic acid, found that OPP was slightly more potent than tea, at equivalent phenolic concentrations (20 and 100 ppm), but was more potent than gallic acid and catechin at concentrations up to 500 ppm GAE (Sambanthamurthi *et al*., 2005).

Supplementing the skin with additional antioxidants has been demonstrated to give additional protection from sun-induced damage; slow down skin ageing, reduce inflammation and ultimately improve skin appearance. Hence, topical antioxidants are now recognised as an integral part of a comprehensive sun protection programme and a valuable addition to any anti-ageing skin care regimen. As such, topical antioxidants have far-reaching benefits for protecting and improving UV-damaged and ageing skin (Chiu *et al*., 2003; Pinnell, 2003). Hence, OPP has a great potential as an antioxidant for topical application. Being a new ingredient, its safety must however be substantiated prior to its use as an active for consumer protection and to meet regulation requirements.

MATERIALS AND METHODS

Materials

OPP was obtained from the Malaysian Palm Oil Board (MPOB), while a commercial green tea extract (ABS Green Tea Extract) was purchased from Chemical Solution Sdn Bhd, Malaysia. The commercial green tea extract was used as a benchmark active for comparison purposes. *Table 1* shows the compositional breakdown of ABS Green Tea Extract.

Methods

In vitro *ocular and dermal irritection assay.* Any substance or ingredient that induces corneal or dermal irritancy is related to its tendency to promote denaturation and disruption of corneal proteins or induce alterations in the structure of keratin, collagen and other dermal proteins. The ocular or dermal irritection assay is an alternative method to animal irritancy studies (Draize Test) that mimic biochemical phenomena. The *in vitro* irritection assays have been reported to correlate with *in vivo* irritancy tests (Sina *et al.,* 1995).

Ocular or dermal irritection assays, consist of two essential components: a membrane disc that permits controlled delivery of the test material to a reagent solution and a reagent solution that is composed of proteins, glycoproteins, lipids and low molecular weight components that self-associate to form a complex macromolecular matrix. Controlled mixing of the test material and the reagent solution during the assay incubation period promotes protein denaturation and disaggregation of the macromolecular matrix. The changes in protein structure that were induced by the test material was readily quantitated by measuring the resulting changes in turbidity at 405 nm (OD_{405}) of the reagent solution for ocular, while in dermal irritection assay, the extent of dye release and protein denaturation was quantitated by measuring the changes in optical density of the reagent solution at 450 nm (OD_{450}).

The application of an irritant chemical to the membrane disc disrupts the ordered structure of keratin and collagen, and results in the release of the bound indicator dye. A comparison of these optical density measurements to those produced by standard chemical irritants permits the calculation of an 'irritancy score' that has been shown to be directly

TABLE 1. COMPOSITIONAL BREAKDOWN OF ABS GREEN TEA EXTRACT

Ingredients	%
Water	35.00-45.00
Propylene glycol	35.00-45.00
Camellia Sinensis (Green Tea) Extract	15.00-25.00
Phenonip	0.80-1.20
EDTA	0.08-0.12

related to the potential corneal or dermal irritancy of the test material. The ocular irritancy potential of a test sample is expressed as an Irritection Draize Equivalent (IDE), whereas the dermal irritancy potential of a test sample is expressed as a Human Irritancy Equivalent (HIE). IDE and HIE have been reported to correlate with *in vivo* investigations by the Draize method and human test respectively. The predicted *in vivo* classification based on these scoring systems, are shown in *Tables 2* and *3* respectively.

For ocular or dermal irritection assays, a standard volume dependent dose-response cosmetic protocol was performed. The following doses of neat sample were applied for analysis: 50, 75, 100 and 125 μl and placed onto membrane discs. Reagent and blank buffer were added to a 24-well assay plate. The assay plate was incubated at 25°C for 24 hr. The membrane was then removed from the assay plate and 250 µl of reagent and buffer were transferred to a 96-well reading plate. The plate was then inserted into a plate reader (MRX Microplate Reader, Dynex Technologies, Inc., Chantilly, VA, USA), which reads the optical density of the respective samples.

In vivo Patch Test

A paper disc saturated with OPP at 1%, 3%, 5% and 100% respectively were placed in Finn Chambers aluminium cells with a diameter of 8 mm. The cells were put on plaster strips, Scanpor (2 x 5 cells per strip) of 60 cm^2 area. All samples were put directly into the cells and applied on the back of human subjects. For each new subject, the patches were randomly positioned on one site to reduce site-to-site variation. The application of the Finn Chambers was made immediately after filling. After the application of plaster strip, the cells were pressed upwards in order to press out the air from the Finn Chambers and make the plaster adhere to the skin. Another plaster was then applied to ensure adhesion of the cells. At Day 3 or 48 hr after the application of the patches, they were removed and subjects were required to relax for 30 min prior to skin reaction reading. This was to eliminate all possible skin redness due to plaster strips occlusion. Subjects were required to return for readings 48 and 96 hr after patch removal. The reactions were scored according to the skin reaction values stated as in *Table 4*.

In vivo Human Repeated Insult Patch Test

A paper disc saturated with OPP and green tea at 1%, 3%, 5% and 100%, formulae with 5% actives (OPP and green tea) were placed in Finn Chamber while an empty Finn Chamber was used as negative control. Finn Chambers of 8 mm diameter with 20 µl filling volume were used in the study. The samples and negative control were applied under occlusive patches to skin sites on the scapular back. Daily

TABLE 2. CLASSIFICATION OF IRRITATION DRAIZE EQUIVALENT SCORE TO *in vivo* IRRITANCY CLASSIFICATION (Draize Test)

Irritection Draize Equivalent score	Predicted Ocular Irritancy Classification
0.0 – 12.5	Minimal irritant
12.5 – 30.0	Mild irritant
30.0 – 51.0	Moderate irritant
> 51.0	Severe irritant

TABLE 3. CLASSIFICATION OF HUMAN EQUIVALENT SCORE TO *in vivo* IRRITANCY CLASSIFICATION

Human Irritancy Equivalent	Predicted Dermal Irritancy Classification
0.00-0.90	Non-irritant
0.90-1.20	Non-irritant/irritant
1.20-5.00	Irritant

TABLE 4. EVALUATION OF SKIN REACTION AND SCORING CRITERIA FOR PATCH TEST

Reaction description	Score
No erythema	-
Light faint homogenous erythema or 'silk paper' skin	+/-
Faint homogenous erythema	+
Evident erythema, bright red, sharp outline	++
Erythema and bulla, follicular bulla and postules	+++
Necrosis	++++

reapplications of the same test material were made on the same test site for 21 days, or until irritation scores of 3.0 or greater were observed. The patches were removed 24 hr after each application. The test site was carefully examined for irritation, scored and then re-patched with fresh test material. In cases where re-application of the test material was discontinued because of the severity of the irritation, scores would be carried through to the end of the induction phase. Each subject was instructed to keep all patches as dry as possible and to remove and discard them at approximately 24 hr. Patch removal was carried out half an hour prior to grading. A rest period of two weeks following the induction phase was carried out. After the rest period, a single challenge patch test was carried out on the back at a different site for 48 hr. The readings were made 48 hr and 96 hr after the removal of the patch. The score readings and classifications were based on modified 21 days cumulative irritation tests according to Berger and Bowman (1982) as shown in *Tables 5*, *6* and *7*.

A score of 3 or higher is assigned a maximum score of 3. If the reaction with a score of 3 or more persists in subsequent days, then the test is terminated and the subject gets a maximum score of 3 for the rest of the study. A persisting reaction of score > 1 on the challenge site may be indicative of irritancy. Allergic responses normally do not improve markedly between 72 to 96 hr. Oedema or infiltration which persists or increases in intensity is suggestive of an allergic response. Other indicators are flare-ups at former application sites, which develop between the induction and the challenge phase. Total cumulative scores were then calculated and classified according to Berger and Bowman Classification of Cumulative Irritation as shown in *Table 7*.

RESULTS AND DISCUSSIONS

In vitro Ocular Irritection Assay

The evaluations were carried out using OPP at 1%, 5%, 50% and 100% concentrations respectively in comparison to commercial green tea at the same concentrations using the volume dependent cosmetic protocol. *Figure 5* shows the *in vitro* ocular irritation potentials of the samples tested. Green tea at 100% (as it is) was found to have scores of between 14.7-28.8 which fell into the category of mild irritant, while OPP had much lower scores between 6.7-13.1,

TABLE 5. SCORE OF REACTIONS OF TEST MATERIAL DURING THE INDUCTION PHASE

Reaction description	Score
No irritation	0
Minimal redness, barely perceptible	1
Definite erythema, readily visible, minimal oedema/popular	2
Erythema and papule	3
Definite erythema	4
Erythema, oedema and papule	5
Vesicular eruption	6
Strong reaction spreading beyond test site	7

TABLE 6. SCORE OF REACTIONS OF TEST MATERIAL DURING THE CHALLENGE PHASE

Reaction description	Score
No reaction	0
Macular erythema	0.5
Indurated erythema	1.0
Erythema, infiltration and redness	2.0
Bullous reaction or ulcer	3.0

TABLE 7. CLASSIFICATION OF OBSERVED RESPONSE

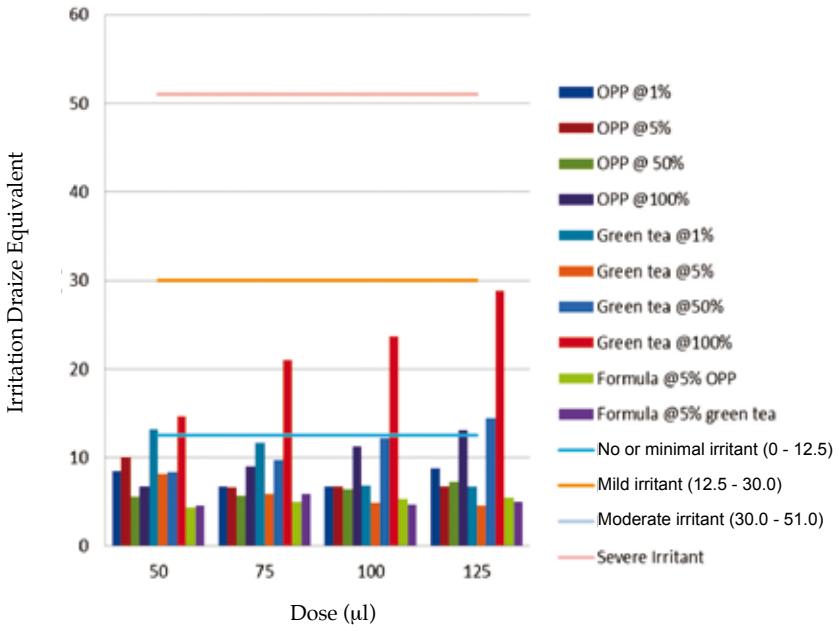
Category	Total cumulative score	Conclusion from test	Description of observed response
1	0-69	Mild material – no experimental material irritation	Essentially no evidence of cumulative irritation under condition of test (*i.e.* conditions at concentrations specified)
2	70-276	Probably mild in normal use	Evidence for slight potential for very mild cumulative irritation under conditions of test
3	277-621	Possibly mild in normal use	Evidence of moderate potential for mild cumulative irritation under conditions of test
4	621-805	Experimental cumulative irritant	Evidence of strong potential for mild to moderate cumulative irritation under conditions of test
5	805-874	Experimental primary irritant	Evidence of potential primary irritation under conditions of test

Source: Berger and Bowman (1982).

which fell into the category of no or minimal irritant. The formulae containing 5% OPP and green tea were classified as no or minimal irritant. Moderate and severe irritant chemicals begin with IDE scores of 30 and 51, respectively. Thus, the *in vitro* ocular assay predicted that OPP had no or only minimal irritation potential, while green tea had a mild irritation potential.

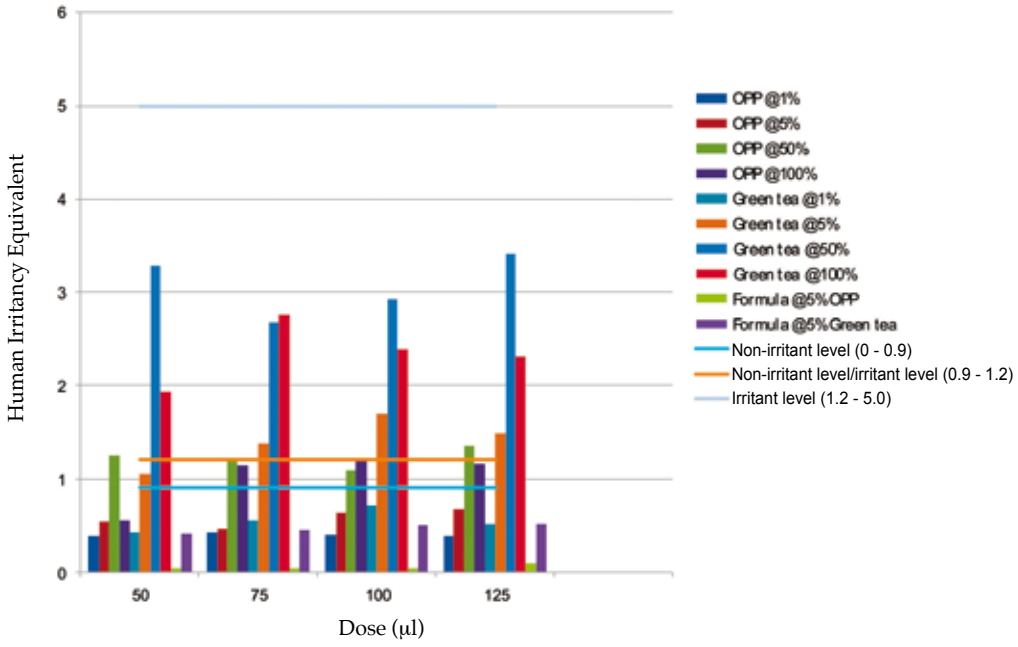
In vitro Dermal Irritation Assay

Figure 6 shows the results of *in vitro* dermal irritation potentials of OPP in comparison to commercial green tea. Both OPP and green tea at 50% concentrations and above were classified as irritant. However at 5% concentrations, green tea was classified as irritant, while OPP was classified as non-irritant. Both formulae containing 5% of the ingredients were classified as non-irritant. It was observed that the dark brown colour of the ingredients might have interfered with optical density measurements used to predict the 'irritancy scores' in comparison to those produced by standard chemical irritants which were shown to be directly related to the potential corneal or dermal irritancy of the test material.



Note: OPP - oil palm phenolics.

Figure 5. In vitro *ocular irritation potential.*



Note: OPP - oil palm phenolics.

Figure 6. In vitro *dermal irritation potential.*

In vivo Patch Test

For the *in vivo* patch test, the ingredients were assessed at 1%, 3%, 5% and 100% concentrations to further confirm on the irritation potential predicted by the *in vitro tests*. In this study, the paper disc was dipped into OPP and green tea at the tested concentrations. The samples were patched on the back of 20 subjects, all males, aged between 23 and 49 years old (average age 37.4 years), in good health and free from skin infections. The samples were patched for 48 hr and clinical evaluation done after the removal of the patch. OPP and green tea at 1%, 3%, 5% and 100% concentrations did not induce any irritation reactions after 48 and 96 hr of patching. Similar observations were also recorded for the empty Finn Chamber.

In vivo Human Repeated Insult Patch Test (HRIPT)

HRIPT was used to determine the incidence and severity of cumulative irritation and allergic contact dermatitis by use of the predictive patch test techniques. Repeat patching of the test material had been shown to produce both cumulative irritation and allergic contact dermatitis. A total of 20 subjects, eight females and 12 males, aged between 28 and 54 years (average age: 40.05 years), of good health and being free from skin diseases, were included in the study. They were clearly informed of the study and the possible risks related to it. However, re-application of OPP and green tea at 100% concentration were discontinued on two subjects on the second week because of the severity of the irritation with scores of 3 respectively. The scores were carried through to the end of the induction phase. Therefore, the total cumulative scores for 100% OPP and 100% green tea were 98 respectively (*Table 8*), which were in Category II of the Berger and Bowman Classification. These samples at 100% concentrations have slight potential for very mild cumulative irritation and therefore were not suitable for topical application. After the induction phase, all the test samples, *i.e.* OPP and green tea at 1%, 3%, 5% and formulae with 5% actives showed total cumulative scores between 0-27 (*Table 8*) which were in category I of the Berger and Bowman Classification. These materials were therefore mild as there was no evidence of cumulative irritation.

After the induction phase, the test subjects were rested for two weeks. A single challenge patch with OPP and green tea at 1%, 3%, 5%, 100% and formulae with 5% active ingredients were then applied on a different site for 48 hr and the readings taken 48 hr and 96 hr after the removal of the patch. The reactions were transient at 48 hr and 96 hr (*Table 9*). Most allergic responses normally do not improve markedly between 72 to 96 hr. However, the challenge patch of OPP and green tea at 1%, 3%, 5% and 100% and formulae with 5% active ingredients did not record any significant cutaneous reactions. Therefore, the results indicated that OPP at 1%, 3% and 5% did not cause contact dermatitis or cumulative skin irritation and were comparable to the commercial green tea extract.

TABLE 8. INTERPRETATION OF TOTAL CUMULATIVE SCORES FOR OIL PALM PHENOLICS (OPP) AND GREEN TEA AT 1%, 3%, 5%, 100% AND FORMULAE WITH 5% ACTIVES AND BLANK FINN CHAMBER

Sample	Total cumulative score	Classification	Conclusion
Blank Finn Chamber	0	1	Mild
OPP 1%	0	1	Mild
OPP 3%	4	1	Mild
OPP 5%	6	1	Mild
OPP 100%	98	2	Probably mild
Formula with 5% OPP	6	1	Mild
Green tea 1%	4	1	Mild
Green tea 3%	4	1	Mild
Green tea 5%	27	1	Mild
Green tea 100%	98	2	Probably mild
Formula with 5% green tea	6	1	Mild

TABLE 9. RESULTS OF THE CHALLENGE PATCH FOR OIL PALM PHENOLICS (OPP) AND GREEN TEA AT 1%, 3%, 5%, 100% AND FORMULAE WITH 5% ACTIVES

Reaction score	Reaction at 48 hr			Reaction at 96 hr		
	0	0.5	1	0	0.5	1
OPP 1%	20	0	0	20	0	0
OPP 3%	19	1	0	20	0	0
OPP 5%	19	1	0	20	0	0
OPP 100%	16	3	1	20	0	0
Formula with 5% OPP	20	0	0	20	0	0
Green tea 1%	20	0	0	20	0	0
Green tea 3%	19	1	0	20	0	0
Green tea 5%	18	2	0	20	0	0
Green tea 100%	18	1	1	20	0	0
Formula with 5% green tea	20	0	0	20	0	0

CONCLUSION

The safety evaluation of OPP was conducted and compared with a commercial green tea extract as a benchmark. The *in vivo* safety evaluation of OPP and commercial green tea extract at 1%, 3% and 5% and formulae containing 5% actives did not produce any irritation or sensitisation, even though the *in vitro* dermal test predicted green tea at 5% as irritant, while OPP as non-irritant. The dark brown colour of the ingredients might have interfered with the optical density measurements used to predict the 'irritancy scores' and hence influenced the results of the *in vitro* tests. Based on the *in vivo* tests, therefore it can be concluded that OPP as an active ingredient for topical application at 5% and below can be considered safe for use. The results are comparable to green tea, a commercially available active ingredient used for cosmetic and personal care applications.

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