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Assessment of antioxidant capacity, total phenolic and flavonoid contents in the agricultural fruit Matad (*Dillenia indica* L.) for hair care applications

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Abstract The phytochemical profile, antioxidant potential and formulation stability of fruit extract of *Dillenia indica* L. (Matad) with emphasis on its potential use in hair care products were investigated. The fresh fruits from Koh Kret had moisture of 61.54%. 1921 maceration with ethanol. The extraction of 20 g of fresh fruit gave 738.85 g of dried material and 35.48 g of extract. The extraction yield was 1.84% (w/w) based on fresh weight. The DPPH radical scavenging assay was used for the antioxidant activity and the result showed concentration dependent response. The IC50 values of extract and ascorbic acid were 2.94 ± 0.12 mg/mL and 0.043 ± 0.003 mg/mL respectively. Importantly, the phytochemical analysis revealed high levels of total phenolic (397.5 ± 4.6 mg gallic acid equivalents/g extract) and flavonoid content (242.0 ± 5.1 mg quercetin equivalents/g extract). These bioactive constituents are responsible for a large part of the antioxidant potential. Extra stability testing helped to prove the product was viable. The shampoos and conditioners with the extract had stable organoleptic properties and pH values for 28 days at different storage temperatures. Centrifugation confirmed that the conditioners did not undergo phase separation. *D. indica* was found to be a promising natural, eco-friendly ingredient for hair care with the high concentrations of phenolics and flavonoids, the measurable antioxidant capacity and the stable formulations despite the modest yields. Future studies are needed to evaluate in vitro and in vivo bioactivity, optimise extraction techniques and to perform consumer acceptability testing for wider sustainable cosmetic innovation.

Keywords: *Dillenia indica*, Antioxidant, Phenolic content, Flavonoid content, Koh Kret

Introduction

Natural products are important sources of bioactive compounds for the pharmaceutical, food production and cosmetic industries. Agricultural plants are scientifically studied more because they contribute to human health, nutrition and personal care (Akhtar *et al.*, 2019). Fruits are characterised by the presence of high amounts of secondary metabolites such as phenolic acids and flavonoids,

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which exert remarkable antioxidant activities and associated biological effects (Saikumar and Badwaik, 2025). Antioxidants are essential in fighting off free radicals, combating oxidative stress and preventing cell damage that causes chronic diseases, premature ageing and damage to skin and hair health. In the cosmetic industry, plant-based antioxidants are gaining popularity in the formulation of hair care products owing to their protective effects on hair follicles and scalp from environmental toxins, ultraviolet (UV) radiation, and chemical exposure. The growing demand for safe, natural and sustainable botanical ingredients reflects the wider trend of green cosmetics and innovation in sustainable agriculture (Kowalczyk *et al*., 2024).

Dillenia indica L., elephant apple, known as "Matad" in Thailand (Figure 1), is a tropical fruit native to South and Southeast Asia. It has been used for local diets, medicine and farming for generations. It has been used to help metabolic and digestive problems and fevers (Abdille *et al*., 2005; Boparai *et al*., 2016; Nayak *et al*., 2020; Choudhury *et al*., 2024). *D. indica* is used in personal care other than medicine. Mucilage of the fruit is used for promoting hair growth and its extracts are used for treatment of dandruff and hair loss. These traditional uses have been further supported by modern research which has found the fruit to contain phenolic acids, flavonoids and terpenoids. The natural compounds possess antioxidant, antimicrobial and anti-inflammatory properties that helps in maintaining health of the scalp and supports the hair growth (Saiful and Armania, 2014; Sidiq and Fauzi, 2023). Although it has diverse traditional uses and a promising phytochemical profile, there are still limited systematic scientific evaluations of *D. indica* with respect to hair care applications. However, research on its antioxidant potential, total phenolic content (TPC) and total flavonoid content (TFC) are especially limited. Nevertheless, such evaluations are essential to prove its potential as a functional ingredient in cosmetic formulations. TPC and TFC are commonly used as reliable parameters to estimate the antioxidant capacity of plant extracts (Saikia *et al*., 2023; Khatun and Sen, 2025). Phenolic compounds and flavonoids have radical scavenging, metal chelation and UV-protective activities and are important for cellular homeostasis. These compounds also have antimicrobial activity that helps to balance the scalp microbiota and anti-inflammatory activity that may help to reduce irritation and promote follicular health. Evaluation of these parameters in *D. indica* would not only provide evidence for incorporation in hair care products but also extend the knowledge of underutilised agricultural plants with high functional potential.

Figure 1. Appearance of Matad: A, stem; B, leaves; C, flower; D, fresh fruit; and F, fruit section showing pulp and seed

Increased demand for natural and sustainable cosmetic ingredients has increased interest in agricultural plants as sources of bioactive compounds. Concerns about toxicity, skin irritation and environmental persistence of synthetic additives have accelerated the shift to botanical alternatives which offer safety, efficacy and environmental compatibility. Agricultural crops rich in phytochemicals are presented as valuable resources for the cosmetic industry, contributing to biodiversity conservation and sustainable agriculture and supporting product innovation (Estarriaga-Navarro *et al*., 2025). The Matad fruit, known in traditional hair care and for its antioxidant properties (Abdille *et al*., 2005; Choudhury *et al*., 2024), is an example of a crop linking ethnobotanical knowledge with modern cosmetic science. Matad fruit is grown in the rural areas of South and Southeast Asia, including Thailand, but it is still underutilised commercially, despite its contribution to local livelihoods (Islam *et al*., 2013).

Assessing its bioactive potential for cosmetic applications can create economic opportunities for small-scale farmers, strengthen rural economies, and facilitate the integration of traditional crops into modern industries (Mohanta *et al*., 2024). In Thailand, Koh Kret in Nonthaburi Province is known for its cultural heritage and herbal products (Phattanawasin, 2009), but the Matad fruit is not widely exploited. The finding of suitability for cosmetic formulations could spur innovation locally and expand market opportunities.

The antimicrobial, anti-inflammatory and gastroprotective properties of *D. indica* have been reported earlier (Saikia *et al*., 2023; Khatun and Sen, 2025) but its application in personal care, particularly hair care, is less explored. A systematic evaluation of the phytochemical profile and antioxidant activity with respect to other plants used in cosmetics is required to validate its efficacy. In this study, the antioxidant activity, total phenolic content and total flavonoid content of Matad fruit from Koh Kret, Thailand were studied to evaluate the potential use as a natural cosmetic ingredient.

Materials and methods

Study area and sample collection

Fresh fruits of Matad were collected during the peak fruiting season (March–May) from Koh Kret (13°91′24″ N, 100°48′69″ E), Nonthaburi Province, Thailand. The area is characterized by a tropical monsoon climate with fertile alluvial soils, which may influence the phytochemical composition of local plant species. Fruits were harvested at physiological maturity. A voucher specimen was deposited for future reference.

Preparation of matad fruit extract

The fruit samples used in this study were ripe fruit with a bright, creamy color (Figure 1F). Fruits, free from any defects, were selected based on the uniformity in size and shape, ranging from 8 to 10 cm in diameter. Fruits were washed with clean water after the stalks were removed. The fruit was cut into small pieces and then dried at a temperature of 40-45°C for a continuous period of 48 hours, until it was completely dry. This drying procedure was repeated three times to ensure accuracy and consistency of the results. Calculate the percentage of volatile matter and water content in the Matad fruit sample using the following formula. Where W_1 represents the initial weight and W_2 represents the final weight.

$$\text{Water content (\%)} = \frac{(W_1 - W_2)}{W_1} \times 100$$

For extraction, the dried Matad fruit sample was immersed in 95% (v/v) ethanol at a ratio of 1:3 for 48 hours. Following maceration, the extracts were filtered through Whatman® No. 4 filter paper, and the solvent was evaporated using a rotary evaporator (Heidolph) at 45°C to obtain the crude Matad fruit extract (MFE). The MFE was stored in a cool, dark place until further use. The percentage yield (% yield) was calculated using the following formula:

$$\text{\% Yield} = \frac{\text{Weight of Matad extract (g)}}{\text{Dry weight of Matad fruit (g)}} \text{x}100\%$$

Finally, the MFE were stored in amber glass bottles at −20°C before antioxidant and phytochemical assays.

Determination of antioxidant capacity

The antioxidant activity was tested by DPPH (1,1-diphenyl-2-picrylhydrazyl) method as described in Lisdiani et al. (2022) with slight modifications. Absorbance was measured at 517 nm and the experiment was performed in triplicate. A regression curve (concentration vs percentage inhibition) was plotted for each concentration and percentage inhibition of MFE and standard (ascorbic acid) were calculated. From this curve IC_{50} values of extracts and samples were calculated. The IC_{50} represents the effective concentration of the standards or samples which reduced the DPPH absorbance by 50%. The lower the IC50 value the greater the antioxidant activity.

Determination of total phenolic content

The total phenolic content (TPC) of MPC was determined using the Folin-Ciocalteu method (Lisdiani *et al*., 2022). The absorbance was recorded at 765 nm. The phenolic content was determined from the standard curve and expressed as gallic acid equivalent (GAE) in mg/g. Each sample was run in triplicates.

Determination of total flavonoid content

The total flavonoid content (TFC) of MFE extracts was calculated using the aluminium chloride colorimetric method (Lisdiani *et al*., 2022). Absorbance was measured in triplicate for each sample at 510nm. The flavonoid content was calculated from a calibration curve and expressed in mg Quercetin equivalent per μg/mL extract.

Formulation development

The shampoo formulations were prepared using deionised (DI) water (71.5-74.5%) as the solvent, Texapon N 70 (13.6%) as the primary surfactant and cocamidopropyl betaine (4%) as a secondary surfactant to increase the foam stability and reduce irritation. Sodium chloride (6%) was used as a viscosity modifier and Luviquart Ultra Care (0.5%) as a conditioning polymer. Microcare PHC (0.7 %) was used as a preservative and fragrance (0.7 %) was used as sensory attributes The active ingredient in the experimental formulation was the Matad fruit extract (3%) which provided antioxidant and bioactive properties for scalp protection and hair vitality. Each batch was made up to 100% (300g) in total.

The conditioner formulations were prepared with cetyl alcohol (4%) as thickener, cocamide monoethanolamide (7%) as foam booster, Tween 20 (2%) as emulsifier and Dehyquart C4046 (10%) as the principal cationic conditioning agent. Other ingredients were: polyquaternium conditioner (Luviquat Ultra Care, 5 percent), pH adjuster (citric acid, 0.2 percent), preservative (Microcare PHC, 0.5 percent) and fragrance (0.3 percent) for sensory enhancement. The rest of the formulation was deionised water. Two systems were prepared, a control formulation containing only the base ingredients and an experimental formulation in which 4% Matad fruit extract was added to the aqueous phase. Both formulations were brought to a total of 100% (200 g per batch).

Stability tests

pH determination and organoleptic evaluation

Formulations were kept at different temperatures (25 °C, 37 °C, 45 °C and 4 °C) for a period of 28 days. Organoleptic characteristics (colour, odour and appearance) were evaluated at seven-day intervals and pH was determined in triplicate.

Centrifugation test

The formulations were centrifuged three times for 30 min each at 3000 rpm in a CENTRIBIO® centrifuge. This procedure was carried out to evaluate any possible phase separation or change in the composition of the formulation.

Statistical analysis

All experiments were performed in triplicate (n = 3) and data were expressed as mean ± standard deviation (SD). Data were analysed using one-way analysis of variance (ANOVA) and Tukey's post hoc test to identify significant differences between means. Statistical significance was defined as $p<0.05$. All analyses were performed using SPSS software version 25.0 (SPSS Inc., Chicago, IL).

Results

Water content and extraction yield

The Matad fruit was collected from Koh Kret, cleaned, sliced and dried at 40–45 °C for 48 h. The proximate analysis showed a moisture content of 61.54% indicating that there was a loss of weight due to evaporation of water during drying process. Initial fresh weight 192120 g, a total of 738.85 g dried material was obtained and then extracted with ethanol by maceration. The dried extract obtained after the evaporation of the solvent was 1.84% (w/w) with respect to the initial weight of the fresh fruit, corresponding to about 35.48 g of extract.

Antioxidant activity

The antioxidant activity of Matad fruit extract was evaluated by DPPH radical scavenging assay using ascorbic acid as reference standard. Both the samples showed dose-dependent increase in free radical scavenging activity with higher concentrations exhibited greater inhibition. The IC_{50} values obtained were 2.94 ± 0.12 mg/mL for the Matad fruit extract and 0.043 ± 0.003 mg/mL for ascorbic acid. The results showed that the extract has a measurable antioxidant activity but the strength of the extract is much less than that of the pure antioxidant compound ascorbic acid. This difference might be because of the complex phytochemical composition of the extract, which contains multiple bioactive constituents with different antioxidant potentials.

Total phenolic content

The total phenolic content (TPC) of the matad fruit extract was determined using the Folin-Ciocalteu method with gallic acid as a reference standard. The extract showed average absorbance of 0.267, 0.266 and 0.275 for three replicates. Based on the gallic acid calibration curve, the TPC was found to be 397.5 ± 4.6

mg gallic acid equivalents (GAE)/g extract. The extract is a rich source of phenolic antioxidants as indicated by high concentration of phenolic compounds.

Total flavonoid content (TFC)

The total flavonoid content (TFC) of Matad fruit extract was analysed by aluminium chloride colorimetric method using quercetin as a calibration standard. The absorbance values of the extract were 0.114, 0.116 and 0.106 in three replicates. The TFC was found to be 242.0 ± 5.1 mg quercetin equivalents (QE)/g extract by the quercetin calibration curve. Such a high flavonoid content suggested that these compounds contributed significantly to the overall antioxidant capacity of the extract.

Stability tests

pH values and organoleptic evaluation

Shampoo and conditioner products held for 28 days at 25, 37, 45, and 4 °C showed no detectable change in colour, odour, or appearance. The pH of the shampoo formulations was found to be in the range of 5.5 to 6.0, which is conducive for minimisation of damage to the hair fibre and also the pH range in which hydrolysed rice protein is stable (5.0–6.0). Conditioner formulations were prepared to have a pH of 4.5 to 5.0. The optimum pH for conditioners is usually 4.0–4.5 which is near the keratin isoelectric point of 3.7 at which hair ion neutralisation occurs. The vegetable polysaccharide blend used in these formulations was stable in the pH range 4.5–7.0. Hence the vehicle used in the formulation was adjusted to pH 5.0 to maintain the stability as seen in the test values.

Centrifugation test

None of the conditioner formulations exhibited phase separation upon centrifugation test. The shampoo formulations were not subjected to centrifugation as they were a single homogeneous phase.

Discussion

The present study aimed to investigate the phytochemical profile, antioxidant capacity and formulation stability of Matad fruit extract with a focus on its potential application as a cosmetic ingredient in hair care formulations. The proximate analysis indicated high moisture content (61.54%) of fresh fruits which is in accordance with the characteristics of tropical fruits rich in water and volatile matter. The extraction yield of 1.84% (w/w) on a fresh weight basis was relatively modest, but comparable to the yields reported for other phenolic-rich fruits submitted to ethanol maceration. This recovery suggests that even though *D. indica* is not a high-yielding source of crude extract, the phytochemical density, especially in phenolics and flavonoids, may compensate the low yield in terms of biological activity (Abdille *et al*., 2005; Choudhury *et al*., 2024). The antioxidant activity assays indicated that the extract possessed some functional benefits and some limitations. Its IC_{50} value was 2.94 ± 0.12 mg/mL, indicating that it can scavenge free radicals but not as well as pure ascorbic acid (0.043 ± 0.003 mg/mL). This difference is likely due to the complex mixture of bioactive compounds present in the extract, each with different amounts and different radical neutralising abilities. Antioxidant activity is relevant, as extracts rich in phenolics and flavonoids can work synergistically and provide benefits beyond radical scavenging, such as metal binding, UV protection and anti-inflammation. These effects are especially important to scalp and hair health (Mohanta *et al*., 2024; Sidiq and Fauzi, 2023).

Chemical analysis revealed an increase in total phenolics and flavonoids concentration in the extract. The extract contained 397.5 ± 4.6 mg gallic acid equivalents per gram (GAE/g) and 242.0 ± 5.1 mg quercetin equivalents per gram (QE/g) respectively. These levels are comparable to those found in other plant extracts used in cosmetic and nutraceutical products. The high levels of phenolic and flavonoid compounds explain the antioxidant activity of the extract and suggest possible multifunctional benefits. The documented antimicrobial and anti-inflammatory activities of these phytochemicals may help in better scalp protection, reduced microbial colonisation associated with dandruff and reduced irritation (Deka *et al*., 2022). These properties support the inclusion of *D. indica* extract in hair care formulations (Sidiq and Fauzi, 2023).

Stability tests proved successful incorporation of *D. indica* in cosmetic products. The shampoo and conditioner maintained their appearance, odour and colour for 28 days at various temperatures which is an indication that they are stable. The pH of the shampoo was 5.5 to 6.0 which is good for hair care because it helps to protect the hair fibres. The pH of the conditioner was between 4.5 and 5.0, which is above the ideal range, but still in the acceptable range. The change to pH 5.0 was probably useful for product stability and fit the vegetable polysaccharide blend. Centrifugation tests did not reveal any phase separation in the conditioner, which is indicative of the physical stability of these products (Deka *et al*., 2022).

In conclusion, the matad fruit represents a great potential as an agricultural resource for cosmetic innovation. Extraction yields were modest but the high phenolic and flavonoid content provided measurable antioxidant activity and formulations were stable under relevant stress conditions. The results indicated that *D. indica* extract could be an eco-friendly functional ingredient for hair care products to promote scalp health and sustainability in the cosmetic market. Future work should involve in vitro and in vivo bioactivity assessments, optimisation of the extraction methods, and sensory evaluation to confirm consumer acceptance, in order to realise the full potential of *D. indica* for cosmetic applications.

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Conflicts of interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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