Curcumin Nanoparticles Incorporated Kumkumati Topical Agent for Gingival Depigmentation- An in Vitro Assessment of Antioxidant Activity

Dr. P Rahmath Meeral, Dr. Indumathy Muthupandiyan, Dr. Meignana Arumugham Indiran

Department of Public Health Dentistry, Saveetha Dental College and Hospital, Saveetha Institute of Medical and Technical Sciences, India.
Corresponding Author: P Rahmath Meeral, Email: prahmath31@gmail.com.

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Gingival depigmentation, Curcumin, Kumkumati, Nanoparticles, Gingival hyperpigmentation

ABSTRACT
Background: To date, no topical herbal formulations are available for the depigmentation of gingiva. The current study aims to formulate the herbal topical cream with curcumin nanoparticles incorporated into kumkumati oil followed by its assessment of antioxidant activity.
Materials and Method: 0.5mg of curcumin nanoparticles were mixed with one ml of dimethyl sulphoxide in a vortex mixture. To this five ml of kumkumati oil and 5mg of carbapol were added and mixed until the acquisition of creamy consistency. This was tested for antioxidant activity using a DPPH assay.
Result: The DPPH inhibition % of 25 µl, 50 µl, and 100 µl are 35.64 ± 0.32, 41.45 ± 0.44, 84.29 ± 0.56 respectively. This is comparable to the standard ascorbic acid which shows antioxidant activity of 98.79 ± 0.45.
Conclusion: The formulated material is shown to possess an increased antioxidant activity and thus can be used as a supplemental topical cream in post-surgical/laser gingival depigmentation periods to prevent repigmentation.

1. Introduction

The most frequent primary cardiac Gingival hyperpigmentation is usually not present as a medical problem, but many people may consider their pigmented gums to be unesthetic especially by the ones with a "gummy smile" or excessive gingival display while smiling. The harmonious smile not only goes with the shape, size and arrangement of teeth but also with how the teeth color goes with the color of gingiva. The prevalence rate of gingival pigmentation is diverse according to race and country. Previous literature found that the pattern of pigmentation among south Indians as hyperpigmentation occurs in attached gingiva and interdental papilla [1]. Hyperpigmentation can be physiological, pathological, or sometimes can be due to the trauma-induced one. Studies have also found that passive smokers might also acquire from environmental tobacco smoke [2]. The indication for the correction of hyperpigmentation arises from the demand by the person for improved aesthetics. Over time gingival hyperpigmentation is mostly treated by various methods which include periodontal plastic surgical procedures like scalpel surgical technique, bur abrasion technique, cryosurgery, laser surgery, graft method, and some chemical techniques [3]. However, these techniques succeeded in producing depigmentation, these were associated with many disadvantages like slow and painful healing, bleeding, multiple appointments, cost expenditure, etc Repigmentation of gingiva was evidenced from most of the previous literature following depigmentation therapy [3,4]. According to the result of a recent systematic review and meta-analysis, though the laser, especially the diode laser, showed better esthetic outcomes, less pain, faster healing, and patients' preference and satisfaction after treatment, it showed more regimentation at 6-month evaluation [5]. Studies evaluated the efficacy of Vitamin C as a gingival depigmenting agent after surgical scalpel depigmentation and reported satisfactory esthetic results throughout the follow-up [6].

Pigmentation is due to five primary pigments Melanin, Oxyhemoglobin, Melanoid, Carotene, and Reduced hemoglobin while the intensity of pigmentation depends on the epithelization thickness, keratinization, vascularity, and the presence of melanin [7]. One of the mechanisms of depigmentation by topical agents is to scavenge active oxygen and limit oxidative damage to cell membrane structure reported to inhibit melanogenesis [8]. Antioxidant properties are currently extensively studied for various materials, including natural ones, to identify new compounds from natural sources. Among the natural compounds, curcumin has been shown to give promising results in the depigmentation of skin with anti-inflammatory, antioxidant, antimicrobial, hypoglycemic, wound healing, chemopreventive, chemosensitizing, and radiosensitizing properties [8,9]. On the other hand, kumkumati oil is an herbal formulation used widely in Ayurveda which has given an excellent result in producing depigmentation of skin. It possesses crocus sativus, the main ingredient

of this formulation has been found to have a potent anti-tyrosinase effect [10].

Nanotechnology has become a promising addition to the cosmetic industry due to its ability to enhance the properties of cosmetic products in general. Nanoparticles have enhanced stability, sustained release, enhanced solubility, and increased entrapment efficiency [11].

Different formulations from herbal extracts as nanoparticles were used either in the form of various topical applications as depigmenting agents in dermatology, but there is limited documentation on the use of such ones as depigmenting agents for gingival melanin hyperpigmentation. Therefore, this study attempts to analyze the existing evidence on the antioxidant activity of curcumin nanoparticles loaded with kumkumati formulation for melanin depigmentation on the skin and gingiva. The current study aims to assess the antioxidant activity of gingival depigmentation formulation made of curcumin nanoparticles incorporated kumkumati cream.

2. Material and Methods

2.1 Formulation of depigmentation cream

0.5mg of curcumin nanoparticles was mixed with one ml of dimethyl sulphoxide in a vortex mixture. To this five ml of kumkumati oil and 5mg of carbapol was added and mixed under homogenizer mixer and manually until the acquisition of creamy consistency. Figure 1a to 1g illustrates the formulation of test cream.

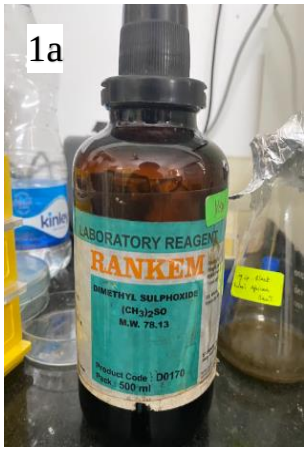
2.2 DPPH assay

DPPH antioxidant activity was measured as suggested by previous study [12]. Briefly, a 0.1 mM solution of DPPH radical solution in 90% ethanol was prepared and 1 ml of this solution was mixed vigorously with 25µl, 50 µl and 100µl of different concentrations of formulated topical cream. After 30 min incubation in the dark and at room temperature, absorbance (*A*) was measured at 518 nm using a UV/VIS spectrometer -T70. The percentage of the radical scavenging activity (RSA) was calculated as inhibition % as % inhibition of DPPH = [Abs control –Abs sample / Abs control] x 100

90% ethanol (1 ml) plus each sample solution (50 µl and 100µl) were used as blank. L-ascorbic acid solution (50 µl at the concentrations of 50-800 µg/ml in ethanol) was used as positive control [i.e., standard/reference].

2.3 Statistical analysis

The results were expressed as mean ± SD (standard deviation) from at least three times measurement. The data were analyzed by the repeated measure ANOVA test using the SPSS 17.0 package (IBM, New York). A probability value of $P < 0.05$ was considered statistically significant.



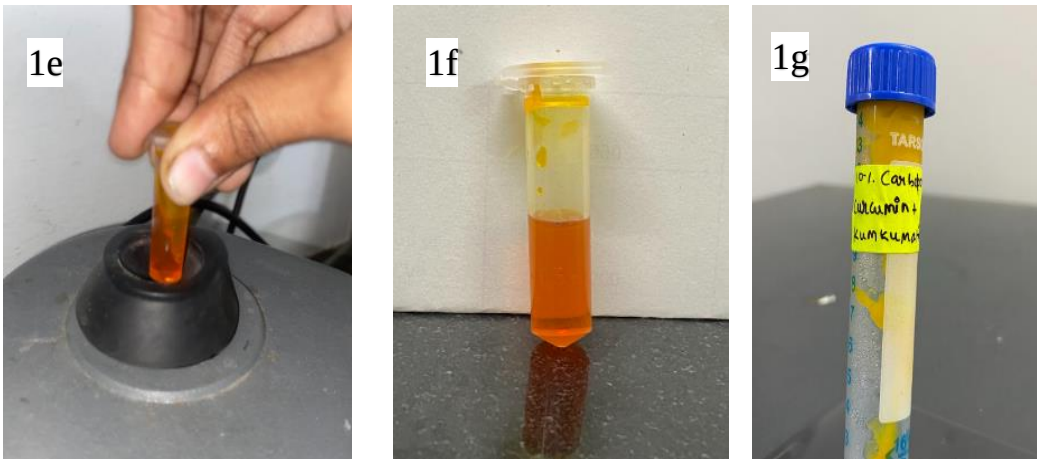


Figure 1. a-g: Formulation of depigmentation cream.

3. Results

The DPPH inhibition % of 25 µl, 50 µl and 100 µl are 35.64 ± 0.32, 41.45 ± 0.44, 84.29 ± 0.56 respectively. This is comparable to the standard ascorbic acid which shows antioxidant activity of 98.79 ± 0.45 which is showed in Table 1 and Figure 2.

Table 1: DPPH inibition% of different concentration of curcumin nanoparticles incorporated kumkumati cream

Test material	Mean DPPH inhibition assay %	p value
25 µl of formulation (n=3)	35.64 ± 0.32	0.005
50 µl of formulation (n=3)	41.45 ± 0.44	
100 µl of formulation (n=3)	84.29 ± 0.56	
50 µl ascorbic acid (n=3)	98.79 ± 0.45	

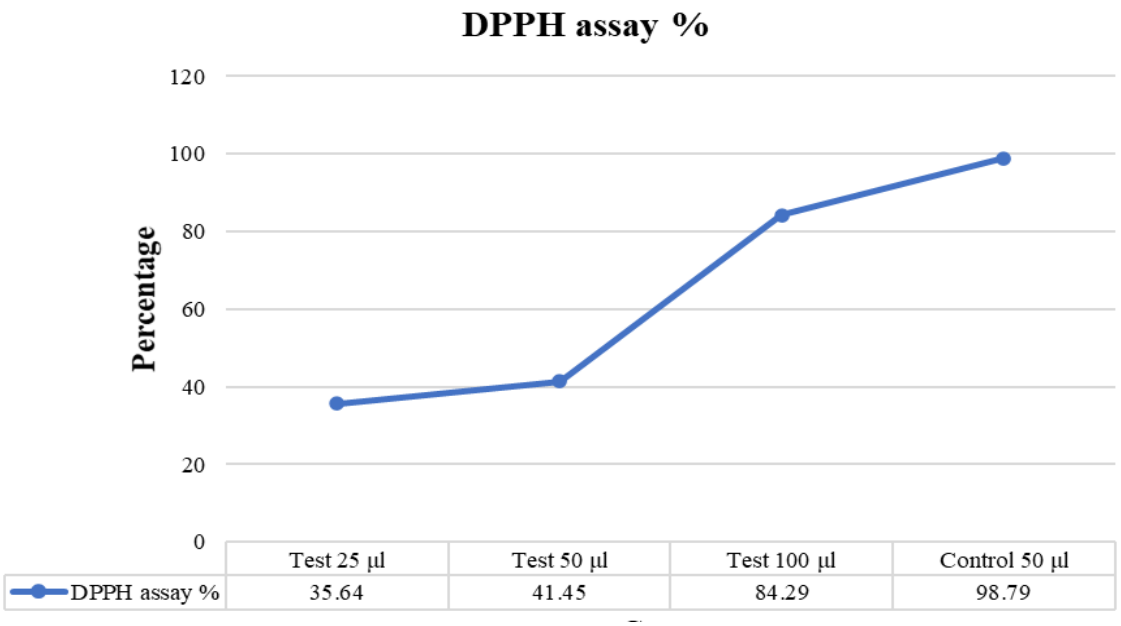


Figure 2. The increase in inhibition % with an increase in the concentration of test formulation

4. Discussion

Curcumin has been the subject of more than 100 distinct clinical investigations, all of which conclusively demonstrate the drug's efficacy, safety, and safety in treating a range of chronic human ailments. The properties of curcumin in various systems, both in vivo and in vitro, have been the subject of several investigations [13]. The anti-inflammatory and antioxidant properties of curcumin have been demonstrated to have several therapeutic effects. It was demonstrated to be an effective scavenger of several reactive oxygen species,

including hydroxyl radicals. Maizura et al. found the DPPH assay % of turmeric extract is 64.6 ± 2.4% [14] Olga et al. estimated ID50 values of curcumin as the highest when compared to ascorbic acid which shows the effective antioxidant activity of curcumin[15]. Qianyun et al investigated the DPPH scavenging capacity of the therapeutic substance increased from 1.81% to 35.16% as the curcumin content increased to 5% [16].

Despite the promising results of curcumin, its color, lack of water solubility, and limited bioavailability severely limit its usefulness. The use of adjuvants, liposomal curcumin, curcumin nanoparticles, curcumin phospholipid complexes, and curcumin reformulated with different oils have all been explored as potential solutions to this problem. On other hand, Kumkumadi oil is an Ayurvedic herbal oil that has been traditionally used in India for various skin conditions, including depigmentation and improving skin complexion. The primary ingredient in kumkumadi oil is saffron, which is known for its antioxidant and skin-lightening properties.

To date, there have been no herbal creams specifically designed and clinically proven for gingival depigmentation. This makes the rationale of the study to utilize the depigmentation effect of curcumin and kumkumati oil in the gingiva. We assumed that there might be a synergistic effect on having antioxidant activity which can cause depigmentation in gingiva. Curcumin as nanoparticles incorporated in kumkumati oil can additionally improve its antioxidant effect which is evident from the result of this current study with DPPH % of 25 µl, 50 µl and 100 µl were 35.64 ± 0.32, 41.45 ± 0.44, 84.29 ± 0.56 respectively which is comparable to ascorbic acid.

Many precious studies have been conducted on the property evaluation of curcumin based products which shows a positive results. Among them, one study by T N Uma et al in 2021 showed insitu gel from turmeric had a significant DPPH antioxidant assay results with 50 µL (90.6.) of gel [17]. The study by Dharman S et al in 2023 showed, Turmeric extracted silver nanoparticles had maximum UV-visible spectrophotometry absorption peak at 440 nm i.e, 88% strong antioxidant with 50 µg/mL [18]. Another study by Dharman S et al in 2023 showed Turmeric extracted gold nanoparticles showed that 90.3% maximum scavenging ability of DPPH at a concentration of 50 µg/mL [19]. Varusha S et al in 2018 showed turmeric oil had good anti-oxidant activity and scavenging activity [20]. Further another study by Bohra et al in 2023 also found that curcumin is known to have greater anti-cancer property with its anti-oxidant activity [21].

Since both curcumin and kumkumati have been known to be used widely, the need for cell vitality and cytotoxicity tests are not necessary. Future studies on the antityrosinase activity of curcumin nanoparticles incorporated kumkumati topical formulation must be done to arrive at a clear conclusion on the depigmentation effect. Also, ex vivo studies with gingival cells can help more to discover the depigmentation effect of curcumin nanoparticles incorporating kumkumati topical formulation.

5. Conclusion

Within the current study's limitations, the curcumin nanoparticles incorporated kumkumati cream were shown to possess antioxidant activity comparable to ascorbic acid in reducing melanin pigmentation. Thus, this cream can be patented and used as a supplemental topical cream following the surgical gingival therapy for depigmentation which helps prevent repigmentation.

However, future exvivo and invivo studies to be conducted to arrive at a promising result on gingival depigmentation without a need of surgical or laser therapy.

References:

[1] Ponnaiyan D, Jegadeesan V, Perumal G, Anusha A: Correlating skin color with gingival pigmentation patterns in South Indians - a cross sectional study. Oral Health Dent. Manag. 2014, 13:132-136.

[2] Sridharan S, Ganiger K, Satyanarayana A, et al.: Effect of environmental tobacco smoke from smoker parents on gingival pigmentation in children and young adults: a cross-sectional study. J. Periodontol. 2011, 82:956-962. 10.1902/jop.2010.100479

[3] Kamboj S, Salaria SK.: Efficacy of liquid nitrogen and electrocautery assisted gingival depigmentation in term of patient's perception, histological wound healing - A randomized triple blind clinical trial. J. Indian Soc. Periodontol. 2020, 24:135-144. 10.4103/jisp.jisp_438_19

[4] Chandra GB, VinayKumar MB, Walavalkar NN, et al.: Evaluation of surgical scalpel versus semiconductor diode laser

techniques in the management of gingival melanin hyperpigmentation: A split-mouth randomized clinical comparative study. J. Indian Soc. Periodontol. 2020, 24:47-53. 10.4103/jisp.jisp_186_19

[5] Gul M, Hameed MH, Nazeer MR, et al.: Most effective method for the management of physiologic gingival hyperpigmentation: A systematic review and meta-analysis. J. Indian Soc. 2019, 23:203-215. 10.4103/jisp.jisp_555_18

[6] Sheel V, Purwar P, Dixit J, Rai P: Ancillary role of vitamin C in pink aesthetics. BMJ Case Rep. 2015, 2015:10.1136/bcr-2014-208559

[7] Subhadarsanee C, Dhadse PV: A Concise Review of Gingival Depigmentation. Techniques and Re-Pigmentation.Indian J Med Forensic Med Toxicol. 2020, 14:6769-6775.

[8] Sathali AH, Ekambaram P, Priyanka K: Solid lipid nanoparticles: a review. Sci Rev Chem. 2012, 2:80-102.

[9] Gopinath H, Karthikeyan K: Turmeric: A condiment, cosmetic and cure. Indian J. Dermatol. Venereol. Leprol. 2018, 84:16-21. 10.4103/ijdvl.ijdvl_1143_16

[10] Mukherjee PK: Validation of medicinal herbs for anti-tyrosinase potential. Journal of Herbal Medicine. 2018, 14:1-16. 10.1016/j.hermed.2018.09.002

[11] Tangau MJ, Chong YK, Yeong K: Advances in cosmeceutical nanotechnology for hyperpigmentation treatment. J. Nanopart. Res. 2022, 24:155. 10.1007/s11051-022-05534-z

[12] Nickavar B, Kamalinejad M, Mohandesi S: Comparison of the components of the essential oils from leaves and fruits of Grammosciadium platycarpum. 2006, 42:686-688. 10.1007%2Fs10600-006-0252-x

[13] Kunnumakkara AB: Curcumin, the golden nutraceutical: multitargeting for multiple chronic diseases. Br. J. Pharmacol. 2017, 174:1325-1348. 10.1111/bph.13621

[14] Maizura M, Aminah A: Total phenolic content and antioxidant activity of kesum (Polygonum minus), ginger (Zingiber officinale) and turmeric (Curcuma longa) extract. Food Research Journal. 2011, 18:529-534.

[15] Pozharitskaya ON, Ivanova SA, Shikov AN, Makarov V.G: Separation and free radical-scavenging activity of major curcuminoids of Curcuma longa using HPTLC-DPPH method. . Phytochem. Anal. 2008, 19:236-243. 10.1002/pca.1035

[16] Ma Q, Ren Y, Wang L: Investigation of antioxidant activity and release kinetics of curcumin from tara gum/ polyvinyl alcohol active film. Food Hydrocoll. 2017, 70:286-292. 10.1016%2Fj.foodhyd.2017.04.018.

[17] TN U, RAJESHKUMAR S. Preparation of Tulasi, Aloe Vera and Turmeric Formulation And Its Antioxidant And Antimicrobial Activity. International Journal of Pharmaceutical Research (09752366). 2020 Jan 2.

[18] Dharman, Sreedevi; Maragathavalli, Gopal; Shanmugam, Rajeshkumar1; Shanmugasundaram, Karpagavalli2. Biosynthesis of Turmeric Silver Nanoparticles: Its Characterization and Evaluation of Antioxidant, Anti inflammatory, Antimicrobial Potential Against Oral Pathogens an In vitro Study. Journal of Indian Academy of Oral Medicine & Radiology 35(3): p 299-305, Jul–Sep 2023. | DOI: 10.4103/jiaomr.jiaomr_309_22

[19] Dharman S, Maragathavalli G, Shanmugam R, Shanmugasundaram K. Curcumin mediated gold nanoparticles and analysis of its antioxidant, anti-inflammatory, antimicrobial activity against oral pathogens. Pesquisa Brasileira em Odontopediatria e Clínica Integrada. 2023 Nov 27; 23:e220068.

[20] Christopher VS, Roy A, Rajeshkumar S. Turmeric oil mediated green synthesis of silver nanoparticles and their antioxidant activity. J. Evol. Med. Dent. Sci. 2018;10(8).

[21] Bohra, Ankita; Maheswari, T. N Uma; Harsh, Aditya1. Evaluation of Antitumor and Antioxidant Activity of Curcuma Caesia Roxib for Targeting Cancer Apoptosis. Journal of Indian Academy of Oral Medicine & Radiology 35(2): p 171-175, Apr–Jun 2023. | DOI: 10.4103/jiaomr.jiaomr_271_22