ANTI-MELANOMA BIO-EFFICACY OF THE PLANT MADHUCA LONGIFOLIA AND ITS ENHANCEMENT USING BIOACTIVE PRINCIPLE LOADED GOLD NANO PARTICLE

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ABSTRACT

Objective: Unexplored in-vivo anti-melanoma bio-efficacy of the plant Madhuca longifolia (bark) has been carried out against C57BL/6 mice.

Methods: Optimized experimental conditions of phytofabrication of gold nanoparticles were as follows: flavonoid content [1 ml, 0.5 mg/ml], sodium tetrachloroaurate dihydrate solution [2 ml, 1 mM], and sonication [15 min, 20 KHz] at pH 4. The optical properties; ultraviolet-visible spectrophotometer (UV-Vis), particles size and zeta potential (Zetasizer), miller indices; X-ray diffraction (XRD), morphology; field emission-scanning electron microscope (FE-SEM), particle size; high resolution-transmission electron microscopy (HR-TEM), surface roughness; atomic force microscopy (AFM) and elemental composition; and energy dispersive X-rays (EDX) of flavonoid loaded gold nanoparticles. In-vivo anti-melanoma bio-efficacy has been carried out against C57BL mice. Radioisotopic, hematological, and histopathological studies were carried out using standard procedures.

Results: Redox potential of the total flavonoid extracted from the bark of the plant (Madhuca longifolia) has been used for the fabrication of flavonoid loaded gold nanoparticles (F@AuNp) and confirmed for the first time their significant anti-melanoma bio-efficacy. The finding is supported by hematochemical and histopathological studies carried out in the organs (liver, kidney, intestine) of C57BL mice. The significant enhancement in phytofabricated F@AuNp compared to native bark extract of the plant has been assigned to enhanced stay period and nanosizing, biocompatibility, nontoxic nature, and enhanced beneficial payload to the cancerous cells.

Conclusion: Such phytofabricated gold nanoparticles possess an admirable prospect for the expansion of herbal nanomedicine for anti-melanoma bio-efficacy.

Keywords: Madhuca longifolia, Flavonoid, Gold nanoparticles, Enhanced bio-efficacy.

INTRODUCTION

Recent realization that folk plants indicated for various bio-efficacies should be established scientifically and to make the use of their active template molecules in biomedical research. Plant secondary metabolites such as vinblastine, vincristine, etoposide, teniposide, taxanes, camptothecin, irinotecan, and topotecan have been well recognized as antioxidants and anticancer bio-agents for direct inhibition of cell proliferation [1-5]. The meagre in-vivo bioavailability of plant secondary metabolites [6] owing to their rapid and extensive metabolism has directed research efforts toward the enhanced accumulation of phyto-modalities in the tumor cells with no or minimum potential toxicity to healthy cells [7,8]. Plant-mediated fabrications of noble metal nanoparticles have signified themselves as an important tool for the enhancement in the pharmacological efficacies. Research devotion has been paid toward the use of diverse plant extracts as such or their bioactive constituents for the phytofabrication of metal nanoparticles [9-22].

Madhuca longifolia (Mahua) belongs to the Sapotaceae family and is an Indian tropical tree found in most part of the country like West Bengal, Maharashtra, Madhya Pradesh, Kerala, Gujrat, Orissa, Chhattisgarh, Jharkhand Uttarakhad, and Uttar Pradesh [23-25]. Among the tribe’s society, its different parts are used as folk remedies for skin-related issues, wound healing, swelling, antioxidant, and local liquor formation [26-28]. Phytochemical screening of various parts of the plant Madhuca longifolia has been restricted mainly to qualitative analysis, exploring the presence of polyphenolics, flavonoids, terpenoids, saponins, tannins, alkaloids, glucosides, and large series of plant acids [29]. Quantitative analyses using gas chromatography-mass spectrometry (GC-MS) and high-performance thin-layer chromatography (HPTLC) have shown the presence of a considerable amount of polyphenolics type compounds, confirming the presence of quercetin as flavonoid [30]. However, the presence of a pentacyclic triterpene derivative in a small amount has also been elucidated on the basis of extensive spectroscopic study [31]. The plant lacks detailed quantitative analysis. Madhuca longifolia is a folk plant of our current interest aiming for its nanoscale pharmacological perspectives.

In the perpetuation of our work on phytofabrication of noble nanoparticles for the enhancement of various bio-efficacies [32-37], we have recently explored in-vitro anti-melanoma bio-efficacy of the plant Madhuca longifolia (bark) against mice (B16F10) and human (A375) melanoma cell lines [35]. The present communication for the first time reports in-vivo anti-melanoma bio-efficacy of the plant Madhuca longifolia (bark) against C57BL/6 mice model. The witnessed anti-melanoma bio-efficacy has been successfully enhanced using gold nanoparticles loaded with flavonoids. The proposed flavonoid loaded gold nanoparticles are labeled as (F@AuNp) and used throughout the manuscript. Such medicinally benign active principle loaded gold nanoparticles possess an admirable prospect for the advancement of commendation of herbal Nano-medicine.

METHODS

Extraction and characterization of flavonoid

Microwave-assisted extraction of the plant (bark; 5 g) was carried out in aqueous-alcoholic solution at 200W for 5 min and was used for the quick test of the formation of gold nanoparticles. For a detailed study, cleaned, shade dried, and powdered bark (200 g) was defatted
Fabrication and Characterization of F@AuNp
Optimized experimental conditions of phytofabrication of gold nanoparticles were as follows: Total flavonoid content (1.0 ml; 0.5 mg/ml), sodium tetrachloroaurate dihydrate solution (2 ml; 1 mM), and neutralization (15 min; 20 KHz) at pH 4. The optical properties (UV-Vis spectrophotometer, Lab India, India), miller indices (XRD, Bruker AXS D8 Advance, Germany), surface morphology (FE-SEM, Nova Nano FE-SEM 450, Netherlands), softness (AFM, Nanosurf Easyscan V 1.8, Switzerland), particles size (HR-TEM, Tecnai G2 T 20 ST, Germany) zeta size, zeta potential (Zetasizer, Nano ZS90 model Malvern, Germany), and elemental composition (EDX, Tecnai G2 T 20 ST, Germany) of F@AuNp were measured.

\textbf{In-vivo anti-melanoma activity}

\textbf{Cell culture}
The mice melanoma (B16F10) cell line was obtained from NCCS, Pune, India, and grown in Dulbecco's Modified Eagle's Medium containing antibiotics, L-glutamine (2 mM), and fetal bovine serum (FBS; 10%). Cells were kept at 37±0.2°C, with relative humidity (100%), CO\textsubscript{2} (5%), and air (95%). The culture medium was changed thrice in a week. The single-cell suspension was made using trypsin-ethylenediamine tetra acetie acid. The cell suspension was diluted with media containing FBS (5%) to obtain final density (2×10\textsuperscript{5}cells/ml).

\textbf{Animal experiments}
Random breed, 5–6 weeks old, and 24–28 g body weight (bw) bearing C57BL healthy mice were maintained under controlled standard environmental conditions of the temperature at 25±1°C with relative humidity (55–65%) under dark and light cycle (14 h:10 h). The animals were fed with standard pellet diet and tap water ad libitum.

\textbf{Ethics statement}
The Guidelines of the Committee (CPCSEA) was followed for all the animal experiments against ethical permission No. 1698/P0/a/13/ CPCSEA, Govt. of India.

\textbf{Experimental designing}
Acute oral toxicity test was performed as per the Organization for Economic Cooperation and Development (OECD) guideline 423 (OECD, 2010) on C57BL/6 mice. The animals were randomly divided into six groups of six animals each. Cells [2×10\textsuperscript{5}/ml] in phosphate buffer saline and suspension [200 μl] were maintained and subcutaneously injected into mice on the dorsal side. After the 4\textsuperscript{th} day of injection, the tumor started budding. At the palpable stage of the tumor, the treatments at (mg/kg, bw) of mice were given orally. Cyclophosphamide (reference drug; 150 mg/kg) was intraperitoneally injected every day for 21 days. During the treatment, the size of the implanted tumor was measured at regular time intervals. Group I served as normal control (NC). The remaining animals were inoculated with B16F10 (cells/mice) and further divided into five groups. Group II served as tumor control (TC), while Group III served as a positive control (PC) and treated with the reference drug (cyclophosphamide; 150 mg/kg; bw). Group IV (BE) was treated with aqueous ethanolic bark extract (500 mg/kg; bw). Group V (FC) was treated with flavonoid content (250 mg/kg; bw). Group VI was treated with F@AuNp (150 mg/kg; bw). All the treatments were given orally after 24 h of inoculation and continued for 21\textsuperscript{st} days (once daily). After the last dose, all mice from each group were sacrificed. All the treatments were administered orally through a metal oropharyngeal cannula while the reference drug was given intraperitoneally. Cyclophosphamide, an anti-neoplastic drug frequently used in treating malignancies; therefore, it was used in the present study.

To observe any life-threatening toxicity of the samples and reference drug, body weights of all animals were measured daily during the treatment period. Tumor growth was determined by measuring the diameter using digital Vernier Caliper daily from the very 1\textsuperscript{st} day of the treatment. Tumor volume (in mm\textsuperscript{3}) was calculated [38]. Tumor growth delay was determined by the standard formula: T-C, where T represents median time (in days) required for the tumor’s to reach a volume of 100 mm\textsuperscript{3} and C represents median time (in days) required for the control group to reach the same size [39].

\textbf{Hematological parameters}
The measurement of haematological parameters [haemoglobin, red blood cells, white blood cells, neutrophils, lymphocytes, and platelets counts] were carried out on the first day in blood sample (0.3 ml) taken from retro- orbital plexus in ethylenediaminetetraacetic acid (EDTA) tube. After 24 h of the tumors cell's inoculation, the animals were given as per designed group treatments (once daily for the next 20 days). On the 21\textsuperscript{st} day, animals were sacrificed and the assessments of hemato logical parameters were again conducted.

\textbf{Radioisotopic study}
All the treatments were tagged with radiolabelled Tc\textsuperscript{99} using stannous chloride reduction [40]. The stannous chloride (SnCl\textsubscript{2}, 2H\textsubscript{2}O) (1 mg/ml; 0.1N HCl) and F@AuNp were mixed in a vial containing (100 μCi; 3.7 MBq[Tc\textsuperscript{99}]) procured from the Department of Nuclear Medicine, Jawaharlal Nehru Cancer Hospital and Research Center, Bhopal, India. The reaction mixture was vortexed and kept at ambient temperature. All the treatments tagged with Tc\textsuperscript{99} were administered in mice through the tail vein and scanned using the Brivo NM 615 scanner at different time intervals.

\textbf{Histopathological studies}
The body organs (liver, kidneys, and intestine) were removed and processed overnight for dehydration, cleaning, impregnation in an automatic tissue processor (Sakura, Japan) and fixed in 10% solution of neutral buffered formalin. The organs were embedded in paraffin blocks followed by sectioning (sections of 5 μm) and staining with hematoxylin and eosin. Slides were examined under a light microscope. The alterations compared to the normal structure were registered. The tumors from each group were dissected and subjected to the above-mentioned treatment course and stained using hematoxylin and eosin. The architecture of the sections was examined using a microscope [41].

\textbf{Statistical analysis}
One-way ANOVA (Tukey-Kramer) was used for statistical analysis and results were given as means±SD. The significance of the value was considered at p<0.05 and p<0.01.
RESULTS AND DISCUSSION

Among the plant secondary metabolites, flavonoids are a broad class of polyphenolic biomolecules with numerous hydroxyl groups and are capable to interact with cell membranes and penetrate more or less deep into hydrophobic cellular sites. They have high medicinal potential behaving as strong antioxidants. Antioxidants, as immunity boosters, have also been proved to strengthen the anticancer activity [42-44]. Relevant literature indicates the presence of flavonoid in a substantial amount in the plant Madhuca longifolia. The total phenolics and flavonoids content in aqueous ethanolic extract of the bark was experimentally measured and found in quite a substantial amount (35.94±0.15 mg/g) and (12.15±0.15 mg/g), respectively. The fact has motivated us to quantify the presence of total polyphenolics and flavonoids in the target plant and to study their role toward in-vivo anti-melanoma bio-efficacy.

The detailed LC-MS characterization of flavonoids extracted from the plant (bark), fabrication of gold nanoparticles loaded with total flavonoid content, and their characterization have been published in our earlier publication [35] in which we have also demonstrated nontoxicity of bark extract and flavonoid loaded gold nanoparticles (F@AuNp) toward the normal lymphocytes cells at the doses selected for the in-vitro assessment of anti-melanoma bio-efficacy. However, the relevant information has been summarized as follows.

HPLC-ESI-QTOF chromatogram (Fig. 1) ascertained the presence of seven flavonoids: 3, 6 dihydroxyflavone; m/z 255.06; retention time 13.9 min (15), dihydroquercetin; m/z 301.21; retention time 2.36 min (23), queretin; m/z 303.05; retention time 10.1 min (11), myricetin; m/z 319.04; retention time 7.6 min (8), myricetin 3-O-arabinoside; m/z 451.08; retention time 4.9 min (6), myricetin 3-O-galactoside; m/z 481.09; retention time 4.8 min (5), and dihexosylquercetin; m/z 621.31; retention time 23.6 min (32) along with other peaks of fatty acids.

The strong synergistic reduction potential of a family of flavonoids extracted from the bark of the plant M. longifolia was used for the phytofabrication of F@AuNp. Table 1 presents the complete characterization of phytofabricated F@AuNp at a glance.

Enhancement of stay period

To monitor the stay period of extracted flavonoid and its loaded gold nanoparticles, radiolabelling experiments were conducted using Tc⁹⁹ as a radioactive tracer having photon emission energy (140 KeV) and half-life (6.0058 h). Radioisotopic scintigraphic images of the melanoma tumors exhibit 4 and 6-h stay periods of flavonoid content and radiolabelled F@AuNp, respectively (Fig. 2). After 4 h, native flavonoid content starts dissipating from the target site. The short-stay period of native phytoextract has also been noticed earlier [44,45]. The present study demonstrates an improved stay period of gold nanoparticles loaded with plant flavonoids, finally leading to improved bio-availability.

In-vivo anti-melanoma activity

Noncytotoxic potential of phytotreatments against human lymphocytes cells

The mean body and tumor weights, tumor volume, and delay in tumor growth (21st days) in C57BL/6, mice model of all the experimental group’s negative control (NC), tumor control (TC), bark extract (BE), flavonoids content (FC), flavonoids loaded gold nanoparticles (F@AuNp), and positive control (PC) have been tabulated (Table 2). All the experimental groups were also periodically monitored for any non-specific toxicity such as food and water withdrawal with impaired movement.

A perusal of Table 2 depicts the following orders of target parameters among all the experimental groups: Mean tumor weight: PC < F@AuNp < FC < BE < TC, Mean tumor volume: TC > BE > FC > F@AuNp > PC, and Mean tumor delay time: PC > F@AuNp > FC > BE > TC. The delay time and tumor volume in F@AuNp treated the group almost matches with that of the reference drug, justifying the promising anti-melanoma efficacy of proposed phytofabricated F@AuNp histopathological observations of various target organs (Fig. 3-5) have been precisely depicted as follows.

Intestine

Group I (NC): Microscopic examination of the TS. sections of intestine depicts the normal shape of intestinal layers, and crypt & villi. Group II (TC): Broken serosa and muscularis layers, the appearance of fragmented villi in the coeliac cavity. Group III (PC):

Table 1: Salient features of the characterizations of F@AuNp

| Techniques | Observations | Findings |
|------------|--------------|---------|
| UV-Vis     | λ<sub>max</sub> = 536 nm | Formation of desired nanoparticles (F@AuNp) Indexed to (111), (200), and (220) lattice face-centered cubic. Polydispersed layer. |
| XRD        | Diffraction peaks (2θ) at 38.2°, 44.5°, and 64.7°. | |
| FE-SEM     | Cubic, triangular, and rectangular morphology | |
| HR-TEM     | Particles diameter | Particle size range 12.20-30.28 nm. |
| AFM        | Surface roughness | Average roughness of 14.18 nm. |
| DLS (Zeta potential) | Negative charge | Zeta potential (~31.6) mV. |
| DLS ) (Zeta size) | Range (11.23 to 159 nm) | Z-average 39.76 nm and PDI (0.238) |
| EDX        | Peaks of Au at 0.34, 2.30, and 9.56 KeV | Formation of F@AuNp along with other elements such as C, O, N and Cu |

Fig. 1: HPLC-ESI-QTOF-MS chromatogram depicting the presence of flavonoids
Fig. 2: Scintigraphic images of tumor-bearing animal of all the experimental groups tagged with Tc99.

Table 2: Effect of mean body weight (BW), tumor weight (TW), tumor volume (TV), and tumor delay time (TDT) in various experimental groups

| Groups       | Group I (NC) | Group II (TC) | Group III (BE) | Group IV (FC) | Group V (F@AuNp) | Group VI (PC) |
|--------------|--------------|---------------|----------------|---------------|------------------|---------------|
| Parameters   |              | mean±SD      | mean±SD        | mean±SD       | mean±SD          | mean±SD       |
| BW (g)       | -            | 31.55±1.17   | 30.31±1.15     | 28.28±1.07    | 26.12±1.02       | 23.83±1.21    |
| TW (g)       | -            | 6.12±1.06    | 4.97±1.34      | 4.12±1.23     | 3.75±1.38        | 3.35±0.19     |
| TV (mm³)     | -            | 118.89±2.56  | 38.70±1.89     | 27.99±1.74    | 24.92±1.55       | 20.33±1.12    |
| TDT (days)   | -            | 1             | 3              | 5             | 6                |               |

Each value is mean±SD (n=6), significance at (p<0.05)

Intestine architectural damage ruptured serosa and muscularis layer, inflammation, and fragmented villi in the coelomic cavity. Group IV (BE): Normal shape of intestinal layers, and crypt & villi. Group V (FC): Normochromic cells, intact serosa and muscularis, with inflammation in crypt and villi. Group VI (F@AuNp): A large number of healthy crypt and villi, proper shape of serosa, and muscularis layers.
Liver
Group I (NC): Normal hepatic lobes, cords of hepatocytes with normal sinusoids space, and normal central vein. Group II (TC): Appearance of few inflammatory cells with abundant micro and macro vesicles, loss of the radiating hepatocytes. Group III (PC): Fatty infiltration and central venous congestion (CVC) pronounced and loss of architecture of hepatocytes. Group IV (BE): Appearance of few inflamed cells with a little number of micro and macro vesicles. Group V (FC): Normal hepatocytes and central vein. Group VI (F@AuNp): Hexagonal hepatocytes with activation of Kupffer cells and slight sinusoidal infiltration.

Kidney
Group I (NC): Normochromic renal cells, with normal medullary rays, and renal glomeruli, the flat epithelium lining with the glomerular capsule. Group II (TC): Inflamed Bowman capsule with widely spaced medullary rays and hypochromic renal cells. Group III (PC): Enlarge vascular glomeruli tightly filled with Bowman’s capsule and appearance of widely spaced medullary rays with inflamed renal cells. Group IV (BE): Normal arrangement of renal glomeruli with flat epithelium lining and appearance of normal glomerular capsule. Group V (FC): Well-populated Bowman capsule equally placed medullary rays. Group VI (F@AuNp): Normal renal parenchyma and glomerular with equally placed medullary rays.

It is inferred that there are no marked changes in the normal architectures (NC) of the target organ treated with F@AuNp. However, the reference drug (cyclophosphamide) treated group (PC) demonstrates noticeable damage to the normal architecture of body organs, warning the specific toxic nature of the reference drug. Other chemotherapeutic anticancer agents have also been reported to induce specific toxicity [46,47]. The C57BL/6 mice tumors of all the experimental groups were also subjected to histopathological studies (Fig. 6). Group II (TC): The presence of melanocytic tumor cells, hyper-vascularization with hyperchromic tumor cells. Group III (PC): Presence of few live tumor cells with a high density of necrosis and dysplastic cells. Group IV (BE): Presence of live tumor cells with apoptosis. Group V (FC): A larger number of apoptotic cells. Group VI (F@AuNp): Enhanced number of apoptotic cells with necrosis and inhibition in angiogenesis and micro-vessels.

The above observations demonstrate that among all the phytotreatments, there is a progressive increase in the anti-melanoma bio-efficacy in terms of apoptosis and necrosis, progressing from BE to FC and reaching to the optimum level in F@AuNp. The significant inhibition in angiogenesis and micro-vessels in F@AuNp treatment is the sign of its accumulation in tumor cells. The suppressed angiogenic vascularization has been reported to lead inhibition of tumor cell proliferation and apoptosis [48,49]. Finally, observations on hematological parameters of all the experimental groups of C57BL/6 mice also provide support to the

Fig. 4: T.S. of liver of all experimental groups (a) NC, (b) TC, (c) PC, (d) BE, (e) FC, and (f) F@AuNp

Fig. 5: T.S. of kidney of all experimental groups (a) NC, (b) TC, (c) PC, (d) BE, (e) FC, and (f) F@AuNp
prominent anti-melanoma bio-efficacy of phytofabricated F@AuNp. The changes in hematological parameters induced by all the experimental groups have been summarized (Table 3).

The observed increase in WBC count in phytotreatments is a sign of development of a defense mechanism against malignancy which is in the divergence of the antitumor effect of a chemotherapeutic agent exhibiting a decrease in the WBC count [50]. All the phytotreatments again appear to reduce Hb and RBC to a lesser extent compared to the reference drug. The development of anemia, an issue with most of the cancer chemotherapeutics and is faced as chemotherapeutic agent-induced myelosuppression activity [51]. The perceived increase in neutrophils and platelet counts and a decrease in lymphocytes in all the tumor-bearing animals relate with the promotion the proliferation of cancerous cells [52]. Thus, biocompatibility, tunable optical and electronic properties, and capping of medicinally important secondary metabolites (flavonoid) designate proposed nanoparticles (F@AuNp) a suitable phytoagent to be developed for an upcoming drug.

![Histopathological changes in tumors of all the experimental groups](image)

**Fig. 6: Histopathological changes in tumors of all the experimental groups**

![Plausible mechanism for anti-melanoma activity of F@AuNp](image)

**Scheme 1: Plausible mechanism for anti-melanoma activity of F@AuNp**

| Groups | Hb (g/dl) | RBC (1×10^6/µl) | WBC (1×10^6/µl) | Neutrophils (%) | Platelets (1×10^6/µl) | Lymphocytes (%) |
|--------|-----------|------------------|------------------|-----------------|------------------------|-----------------|
| NC     | 14.0±0.24 | 9.27±0.25        | 6.20±0.47        | 13.4±1.42       | 683.2±32.14           | 88.4±1.14       |
| TC     | 9.4±1.43  | 6.36±1.05        | 75.73±23.14      | 83.4±3.42       | 1128.0±77.30**        | 13.5±3.54**     |
| BE     | 8.1±0.10* | 5.43±0.74**      | 35.01±4.32**     | 63.5±3.21**     | 96.4±72.25            | 31.4±2.54       |
| FC     | 8.4±0.10* | 5.89±0.73*       | 38.61±4.02**     | 58.5±3.21*      | 95.4±75.25*           | 26.4±2.54*      |
| F@AuNp | 9.8±0.18* | 7.43±0.74        | 13.21±2.21       | 43.5±2.21*      | 85.4±20.25            | 21.4±2.14*      |
| PC     | 8.7±1.25* | 5.06±0.72**      | 56.72±12.21**    | 37.4±2.11**     | 795.2±48.32           | 10.6±1.84**     |

Each value is mean±SD (n=6), *p<0.05, ** p<0.01 significant, compared to control.
delivery system. Nanosizing, inert character, and capability to attach with multiple surface components permit such plant composite nanoparticles to go through inside the cells and transport their payloads without eliciting an important immune reaction and any specific toxicity. The exact mechanism of anti-melanoma action and its enhancement through F@AuNp has not been fully elucidated. Based on our experimental findings and pertinent information available, a tentative mechanism has been proposed in a précised schematic fashion (Scheme-1).

**CONCLUSION**

The native extract of the various parts of the folk plant *M. longifolia* has been studied for various bio-efficacies. However, it lacks phytochemical screening and characterization of bioactive principles. The present communication for the first time reports its unexplored anti-melanoma bio-efficacy of native bark extract, extracted total flavonoid (possible bioactive principle characterized using HPLC-ESI-TOF-MS), and flavonoid loaded gold nanoparticles (nanoscale perspective) against C57BL/6 mice. The proposed phytofabricated gold nanoparticles with no sign of any specific toxicity toward human lymphocytes cells with enhanced anti-melanoma bio-efficacy may be proved superior compared to the reference drug (cyclophosphamide).

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**AUTHORS’ CONTRIBUTIONS**

All authors have contributed toward the results, the analytical experiments, numerical simulations, and contributed to the preparation of this manuscript.

**CONFLICTS OF INTEREST**

There are no conflicts of interest.

**AUTHORS’ FUNDING**

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### Table 4: Anti-melanoma bio-efficacy of *M. longifolia*: Position among various plants explored

| Plant Name         | Plant Part     | Dose     | % inhibition | References                  |
|-------------------|----------------|----------|--------------|-----------------------------|
| *Copaifera multijuga* | Seed oil      | 75 µg/ml | 47.12        | Lima et al., 2003 [53]      |
| *Crataegus azarolus* | Leaves        | 400 µg/ml | 77.57        | Mustapha et al., 2015 [54]  |
| *Andrographis nallamalayana* | Leaves    | 100 µg/ml | 80           | Purushotham et al., 2016 [55] |
| *Hymenaea courbali* | Seed          | 50 µg/ml  | 58           | Spera et al., 2019 [56]     |
| *Sorbus commixta* | Fruit         | 100 µg/ml | 83.47        | Jin et al., 2020 [57]       |
| *Madhuca longifolia* | Bark          | 50 µg/ml  | 65           | Yadav et al., 2019 [35]     |
| *F@AuNp*          | Bark Extracted Flavonoid | 15 µg/ml | 85.15        | Yadav et al., 2019 [35]     |

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