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Summary and Conclusion

The present study attempted to utilize naturally available resources in nanoparticle synthesis, cosmetic and tissue engineering applications. In this regard, the first phase of this study focused on screening the plant metabolites, biosynthesis of gold nanoparticles, synthesis of reduced graphene oxide, and synthesis of gold nanoparticles-reduced graphene oxide composite. In phase II, depilatories were formulated using traditional depilatory agents and their testing. In phase III, biodegradable skin substitutes were successfully prepared and tested. The biological activities of prepared samples were done in all three phases. Based on the results and discussion of this study on "**Traditional painless depilatory agents, their nanoparticle-incorporated biodegradable skin substitutes and their potential application in biological activities**" the following conclusions are arrived at.

Phase I

- The tuber portion of *Cyperus rotundus* and the leaves of *Hemigraphis alternata* samples were extracted with different solvents by refluxing method. The pumice stone was extracted using Sonication and Homogenization method.
- The solvent extracts of *Cyperus rotundus* and *Hemigraphis alternata* confirms the presence of alkaloids, flavonoids, sterols, terpenoids, anthraquinones, anthocyanins, proteins, phenols, carbohydrates, and saponins. These are used as capping agents to synthesize nanoparticles and involve different biological activities.
- Gold nanoparticles were synthesized using selected samples under Sonication, Room temperature, Sunlight irradiation and Microwave heating. Effect of concentration variation was studied using the Room temperature method (HAaNP-5 sec), Microwave heating (CRaNP-5 sec) and Sunlight irradiation method (PSaNP–25 min).
- Graphene oxide was reduced using aqueous extracts of *Cyperus rotundus* and *Hemigraphis alternata* by refluxing method. Gold nanoparticles-reduced graphene oxide composite was prepared by treating the equal ratio of AuNPs and rGO.

- The reduction was confirmed by UV-Visible spectroscopy analysis. The SPR band observed was between 500-600 nm for gold nanoparticles, and reduced graphene oxide showed a 250-280 nm band. The important functional groups involved in the reduction were identified using FTIR analysis.
- The particle size of CRaNP (10-30 nm), HAaNP (22-35 nm) and PSaNP (~11 nm) were obtained in XRD and FESEM analysis. Raman spectroscopy results confirmed the formation of few-layer reduced graphene oxides (CRarGO and HAarGO). The thermal stability of CRarGO was detected up to 674˚C and 856°C for HAarGO.
- Antibacterial activity of the synthesized nanoparticles, plant extracts and pumice dispersion showed excellent inhibition potential. Gold nanoparticles show more inhibition potential against *Escherichia coli*, *Staphylococcus aureus*, *Salmonella paratyphi* and *Bacillus subtilis* in a dose-dependent manner.
- The toxicity of the prepared samples was tested on normal cell lines (HEK-293) and skin cancer cell lines (A431) using the MTT assay. Prepared gold nanoparticles using *Cyperus rotundus*, *Hemigraphis alternata* and pumice stone showed 50% cell inhibition at a lower concentration than rGO and AuNPs-rGO composite. In apoptosis, analysis confirms the cell death and nuclear fragmentation in gold nanoparticles treated skin cancer cell lines.

Phase II

- In phase II, naturally available depilatory agents *Cyperus rotundus* tuber and pumice stone were taken up for the depilatory activity.
- Aqueous extract of *Cyperus rotundus*, aqueous dispersed pumice stone and gold nanoparticles incorporated depilatory cream was prepared using the simple grinding method. EDS results revealed no toxic metals present in the prepared samples. No unpleasant odours were observed in prepared samples, and the plant extracts act as a hydrating agent. The prepared depilatory samples possess high emulsifying properties, and it was noted by physical evaluation.
- An *in vitro* depilation tester was fabricated using a simple glass apparatus to measure the depilation time of hair. Commercially available depilatory creams (Std (V) and Std (F)) were used for comparing the results. The prepared depilatories using *Cyperus rotundus*, pumice stone, and nanoparticles increase the depilation activity and the hair breaking time observe between 3-10 min.

- FESEM analysis confirms the depilation activity of the prepared samples on human hair by distorting the keratin structure.
- The prepared samples' antioxidant (DPPH assay) activity results reveal significant dose-dependent activity. Prepared depilatory creams showed the highest inhibition in a lower concentration.
- The antibacterial activity results of the prepared samples against *Escherichia coli*, *Staphylococcus aureus*, *Salmonella paratyphi* and *Bacillus subtilis*, antifungal activity against *Aspergillus fumigatus* and *Aspergillus flavus* reveal the significant microbial inhibition activity in a dose-dependent manner.
- The toxicity of the prepared samples was tested on normal cell lines (HEK-293) and skin cancer (A375) cell lines using the MTT assay. Lower concentrations of prepared samples show minimum toxicity to the normal cell lines. Cell death and nuclear fragmentation were observed for depilatories treated skin cancer cell lines by apoptosis analysis.
- The approximate cost analysis was calculated for prepared depilatory samples. The cost of commercially available depilatory creams is 70-120 Rs (30-60g). In this study, fewer chemicals are used so can provide the blank depilatory samples (60g) for 30 Rs (based on the cost analysis of each constituent used in the preparation with manpower) at the laboratory level. At the industrial level, the cost of the blank is 35-45 Rs. The cost of gold nanoparticle aided depilatories (60g) is increased to 72 Rs in the laboratory and 72-82 Rs at the industrial level. This increase in rate from blank to gold nanoparticles is due to the cost of gold nanoparticles but the prepared samples are less in cost than commercially available depilatory samples.

The results obtained in phase II revealed that the depilatory action of Cyperus rotundus tuber and pumice stone are suitable for cosmetic applications, especially when used to reduce the usage of toxic chemicals in depilatory products.

Phase III

- The skin substitutes were prepared using biodegradable materials and biosynthesized gold nanoparticles.
- With the increase in gold nanoparticle concentration, the tensile and elongation properties decreased. This observation reveals the excellent binding ability of the plant-aided gold nanoparticle with coordinated materials in a dose-dependent manner.
- Less water uptake and water ageing were observed for CRaNP ($SLFgCRaNP_{1-3}$) aided skin substitutes. Higher water uptake and water ageing were observed for HAaNP aided ($SLFgHAaNP_{1-3}$) skin substitutes.
- The 3D optical profilometric results reveal that the hydrophilic nature was decreased in CRaNP-incorporated skin substitutes and increased in HAaNP-incorporated skin substitutes. The TGA results revealed that the prepared skin substitutes possess thermal stability up to 309-311ºC.
- The surface of the CRaNP-treated skin substitute shows a smooth and even texture. The sponge-like texture of HAaNP-aided skin substitutes reveals the capability to hold more water molecules.
- DPPH radical scavenging activity reveals that the prepared skin substitutes possess significant radical inhibition. The antifungal activity of skin substitutes shows significant inhibition activity against *Aspergillus niger* and maximum inhibitions observed for CRaNP (15 mm) and HAaNP (17 mm) aided skin substitutes.
- The cell toxicity studies reveal that prepared skin substitutes possess anticancer properties against A375 skin cancer cell lines and show less toxicity on normal cell lines (HEK-293) in a dose-dependent manner.
- The cell growth studies confirm the biocompatibility of the prepared samples.
- Commercially available skin substitutes are (OrCel, Biobrane, TransCyte, *etc*) high in cost for their minimum size and less availability. The cost of natural and synthetic skin substitutes is ~15,000-35,000 Rs. In this study low cost and easily available biopolymers, plant samples were used. Based on the individual constituent's rate and manpower the approximate rate of the blank skin substitute is <5000 Rs and 5000-10000 Rs for gold nanoparticles aided skin substitutes.

The present study reveals the biosynthesized gold nanoparticles-embedded skin substitutes to give appreciable portraying their tissue engineering and wound healing applications.

An attempt was made to explore the scope, and scientific validation of Cyperus rotundus, Hemigraphis alternata, Pumice stone, and their Nanoparticles in medicinal applications. The present study reports for the first time on the depilatory formulations and skin substitutes' application. The present study suggests utilizing the selected samples and their nanoparticles in future cosmetic and tissue engineering applications.

Future work

- Isolation of essential constituents from the selected samples through various isolation techniques.
- Formulating the mechanism of prepared samples in depilation and skin substitutes application.
- By varying the components and concentration for making depilatory creams like commercially available depilatory creams.
- Improve the method of preparation and testing on formulated samples for commercialization and cost analysis.
- *In vivo* and *in silico* biological studies for prepared samples.