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Original Article

Dentistry Section

Comparison of Free Radical Scavenging Activity of *Ocimum gratissimum* and *Ocimum tenuiflorum* Herbal Formulation and its Mediated Nanoparticles and Nanocomposite: An In-vitro Study

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ABSTRACT

Introduction: Antioxidants play a crucial role in maintaining oral health by counteracting oxidative stress and Reactive Oxygen Species (ROS) to combat dental diseases. The present Investigated the antioxidant properties of an herbal formulation derived from African basil and black tulsi, both individually and in nanoparticle-based formulations. The present study addressed the need for advanced, sustainable antioxidant therapies by exploring the synergistic potential of nanoparticles synthesised via green methods from *Ocimum tenuiflorum* (African tulsi) and *Ocimum gratissimum* (black tulsi) extracts. By combining the bioactive properties of these herbs with the unique capabilities of nanoparticles, the research goals to enhance antioxidant efficacy and offer innovative solutions for oxidative stress-related dental and biomedical applications.

Aim: To evaluate the efficacy of *Ocimum gratissimum* and *Ocimum tenuiflorum* herbal formulation and its mediated nanoparticles and nanocomposite in neutralising oxidative stress and their potential as advanced antioxidant products.

Materials and Methods: The present in-vitro study, conducted between January and June 2023 at Nanobiomedicine lab, Saveetha Dental College and Hospitals, Chennai, Tamil Nadu, India, evaluated the antioxidant properties of a herbal formulation derived from *Ocimum tenuiflorum* (black tulsi) and *Ocimum gratissimum* (African basil) both individually and in nanoparticle-based formulations. The study included herbal extracts with significant phytochemical content and the methodology involved green synthesis of silver (AgNPs) and Zinc Oxide Nanoparticles (ZnONPs) using the herbal formulation, followed by their characterisation and evaluation via 2,2-diphenyl-1-picrylhydrazyl (DPPH), Hydrogen Peroxide (H_2O_2), and Ferric Reducing Antioxidant Power (FRAP) assays.

Results: The herbal formulation exhibited antioxidant activity in all assay when compared to the standard antioxidant (ascorbic acid), which achieved a maximum inhibition of 92.4 at 50 µg/mL. Incorporation into nanoparticle structures led to further enhancement of antioxidant activity, with the nanocomposite demonstrating the highest antioxidant activity among all samples tested, achieving 90.91 inhibition in the DPPH assay, 86.5 inhibition in the H_2O_2 assay, and a FRAP value of 86.81 at 50 µg/mL, compared to ascorbic acid. Due to the use of a single sample per concentration, p-value cannot be computed and the results are demonstrated using bar graphs.

Conclusion: The findings of this study provide compelling evidence of the potent antioxidant properties of the herbal formulation derived from African basil and black tulsi, particularly when incorporated into nanoparticle-based formulations. These results suggest that herbal-nanoparticle combinations could serve as effective alternatives or supplements to traditional antioxidants, offering a natural and sustainable approach to combating oxidative stress.

Keywords: Free radical scavengers, Herbal medicine, Nanostructures, Phytotherapy

INTRODUCTION

Antioxidants play a pivotal role in maintaining oral health by counteracting oxidative stress and Reactive Oxygen Species (ROS) that contribute to oral and dental diseases [1]. Oxidative stress arises from an imbalance between the body's antioxidant defenses and the production of free radicals, which can cause cellular damage within the oral environment. Sources of free radicals in the oral cavity include various factors such as diet, alcohol consumption, smoking, dental treatments, and periodontal diseases [2].

The mechanism of antioxidants involves neutralising free radicals by donating electrons and has shown potential in inhibiting the growth of oral cancer cells, highlighting their therapeutic relevance in dental care [3]. In dental procedures, antioxidants are utilised to scavenge free radicals and promote cellular repair. Green synthesised nanoparticles, especially those derived from plant extracts represent a groundbreaking development in modern dentistry, offering innovative solutions to address diverse oral health challenges [4]. These nanoparticles, including silver (Ag), gold (Au), and iron (Fe) nanoparticles synthesised through eco-friendly methods, exhibit unique properties that surpass traditional herbal treatments for orodental problems [4]. The synthesis of nanoparticles using *Ocimum tenuiflorum* (African basil) and *Ocimum gratissimum* (black tulsi) herbal formulations represents an innovative approach in dentistry, harnessing the synergistic benefits of natural herbs and advanced nanotechnology for enhanced antimicrobial activity against oral pathogens. Two key types of nanoparticles, ZnONPs and Silver Nanoparticles (AgNPs), synthesised with these herbal formulations, have demonstrated promising antimicrobial properties and potential applications in oral health care [5,6].

The present study involves the synthesis of AgNPs and ZnONPs, as well as nanocomposites, using *Ocimum tenuiflorum* (African

basil) and *Ocimum gratissimum* (black tulsi) extracts. These nanoparticles and nanocomposites were then evaluated for their antioxidant abilities using various assays, including the 2,2-DPPH assay, hydroxyl radical scavenging assay, and FRAP assay with the primary objective of assessing the antioxidant efficacy of the herbal formulations and their nanoparticle derivatives.

MATERIALS AND METHODS

This in-vitro study, conducted between January and June 2023 at Nanobiomedicine lab, Saveetha Dental College and Hospital, Chennai, Tamil Nadu, India. evaluated the antioxidant properties of an herbal formulation derived from *Ocimum tenuiflorum* and *Ocimum gratissimum*, both individually and in nanoparticle-based formulations. The study received approval (SRB number: (SRB/SDC/Ph.D/ORTHO-2041/22/015).

Study Procedure

Preparation of herbal formulation: A solution was formulated by precisely combining 1 g of both *Ocimum tenuiflorum* and *Ocimum gratissimum* with 100 mL of distilled water. The mixture was subjected to heating at 60 degrees Celsius for 15-20 minutes using a heating mantle. Subsequent to the boiling process, the mixture underwent gradual filtration through filter paper. The resultant filtrate, which harbored the extract, was subsequently stored for the synthesis of nanoparticles.

Green synthesis of ZnONPs and AgNPs

The green synthesis of ZnONPs and AgNPs was conducted in this research utilising African basil and black tulsi extracts (*Ocimum tenuiflorum* and Ocimum grattissimum) in the presence of a zinc nitrate solution (30 mM in 50 mL distilled water) and a 1 mM silver nitrate solution, respectively. Initially, a controlled source of zinc ions was provided by preparing a zinc nitrate solution. Subsequently, a mixture of 50 mL of African basil and black tulsi extract, known for their rich phytochemical content, was combined with the zinc nitrate solution [7].

For the synthesis of AgNPs, a 1 mM silver nitrate solution was prepared by dissolving silver nitrate in 80 mL of distilled water, followed by the addition of 20 mL of a filtered herbal formulation extract. The resulting mixtures were subjected to centrifugation at 8000 rpm for 10 minutes [7-9].

Green synthesis of silver and zinc oxide nanocomposites (Ag+ ZnONcs): In order to create silver and zinc oxide nanocomposites (Ag+ ZnONcs) in an environmentally friendly manner, equal amounts of 2 mL were combined from the produced pellets of zinc oxide (ZnO) and silver (Ag) nanoparticles. A magnetic stirrer set to 600 revolutions per minute (rpm) was used to perform this amalgamation. The stirring process was maintained for five to six hours [10].

Antioxidant Activity

DPPH assay: The in-vitro DPPH (1,1-diphenyl-2-picrylhydrazyl) assay is commonly employed to assess the antioxidant properties of various compounds, including plant extracts. The reduction of DPPH radicals into DPPH-H, a colourless or light yellow compound, serves as an indicator of the antioxidant's ability to neutralise free radicals.

To create the stock solution, 24 milligrams of DPPH were dissolved in 100 mL of methanol, resulting in a filtrated mixture with an absorbance of approximately 0.973 at 517 nm. Different concentrations of African basil and black tulsi formulation and its mediated AgNPs, ZnONPs, AgNPs+ ZnONCs (10 µg/mL- 50 µg/mL) were then combined with 3 mL of this DPPH solution, and the mixture was incubated in complete darkness for 30 minutes. Subsequently, absorbance was measured at 517 nm [8,9]. The percentage of antioxidant activity was calculated using the formula:

of antioxidant activity={(Ac-As)÷Ac}×100 [7,8]

Where, Ac represents the control reaction absorbance, and As is the testing specimen absorbance

H_2O_2 Assay

Hydrogen Peroxide (H_2O_2) was prepared as a stock solution at a concentration of 3 (w/v). Horse Radish Peroxidase (HRP) was used as the peroxidase enzyme, with a stock solution prepared at a concentration of 1 mg/mL in phosphate buffer (pH 7.4). The substrate solution, containing 4-aminoantipyrine (4-APA) that changes colour upon reduction, and phosphate buffer (pH 7.4) as the buffer solution were also prepared. Control solutions without the test substance were included for comparison.

For the test solution, African basil and black tulsi formulation and its mediated AgNPs, ZnONPs, AgNPs+ ZnONCs was dissolved in an appropriate solvent (Distilled water) to achieve the desired concentration (10 µg/mL- 50 µg/mL). The assay mixture, comprising hydrogen peroxide, HRP, substrate solution, and the test solution, was prepared in a 96-well plate. The uniform final volume in each well was ensured. The assay mixture was then incubated at 37°C for 30 minutes to facilitate the reduction of hydrogen peroxide by the enzyme and the scavenging of hydrogen peroxide [11].

{Percentage of Inhibition=(Absorbance of Control- Absorbance of Test Solution)/ Absorbance of Control)×100} [11].

The percentage of inhibition obtained serves as an indicator of the scavenging efficiency.

FRAP Assay

The FRAP assay is a widely utilised technique for assessing the total antioxidant capacity of biological specimens. In this method, a FRAP reagent is prepared by combining 300 mM acetate buffer (pH 3.6), 10 mM TPTZ (2,4,6-tris(2-pyridyl)-s-triazine) solution in 40 mM HCl, and 20 mM FeCl3.6H2O solution in a 10:1:1 ratio.

To conduct the FRAP assay, varying concentrations (10 µg/mL- 50 µg/mL) of African basil and black tulsi formulation and its mediated AgNPs, ZnONPs, AgNPs+ ZnONCs and standards are dispensed into a 96-well plate in triplicate, approximately 20 µL each. Subsequently, 200 µL of the prepared FRAP reagent is added to each well, and the plate is incubated at 37°C for 30 minutes. The absorbance at 593 nm is then measured for each well using a microplate reader.

The FRAP value for each sample or standard is determined by comparing the absorbance to a standard curve generated using known concentrations of a standard antioxidant, such as trolox.

The FRAP value is calculated using the formula:

FRAP value (µM Trolox equivalents)=(Absorbance of sample/Slope of standard curve)×Dilution factor [12].

STATISTICAL ANALYSIS

Descriptive statistics were used to analyze the data.Due to the use of a single per concentration, p-value was not calculated and the results are demonstrated using bar graphs.

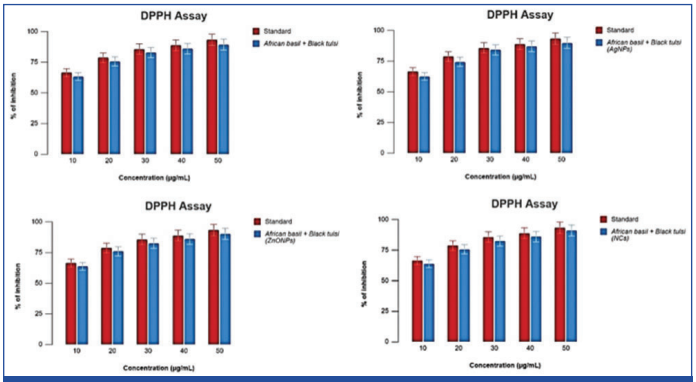
RESULTS

DPPH assay:

The standard marked in the graph is ascorbic acid, a widely used antioxidant. The test groups include the Ocimum-based herbal formulation, AgNPs, ZnONPs, and a nanocomposite combining AgNPs and ZnONPs. The graph highlights the scavenging efficiency of each group, showing that all samples exhibit significant antioxidant activity. Among them, the nanocomposite demonstrates inhibition (91.2) at 50 µg/mL comparable to ascorbic acid (92.4). This suggests a synergistic effect of combining nanoparticles with the herbal formulation for enhanced antioxidant efficacy. Subtle changes may result from small effect sizes, short observation periods, or limited sample sizes.

The results are presented as percentage inhibition of DPPH radical at different concentrations (10, 20, 30, 40, 50 µg/mL) [Table/Fig-1]. The herbal formulation consistently demonstrated significant antioxidant activity compared to ascorbic acid across all concentrations tested (10 to 50 µg/mL). Percentage inhibition values ranged from approximately 63.19 to 91.2, iindicating strong scavenging capability against DPPH radicals.

Incorporation of the herbal formulation for synthesising AgNPs and ZnONPs resulted in enhanced antioxidant activity compared to the formulation alone. Both AgNPs and ZnONPs contributed to increase DPPH radical inhibition, with the nanocomposite formulation exhibiting the highest antioxidant activity among all samples tested, reaching a maximum percentage inhibition of 91.2, at 50 µg/mL [Table/Fig-1].



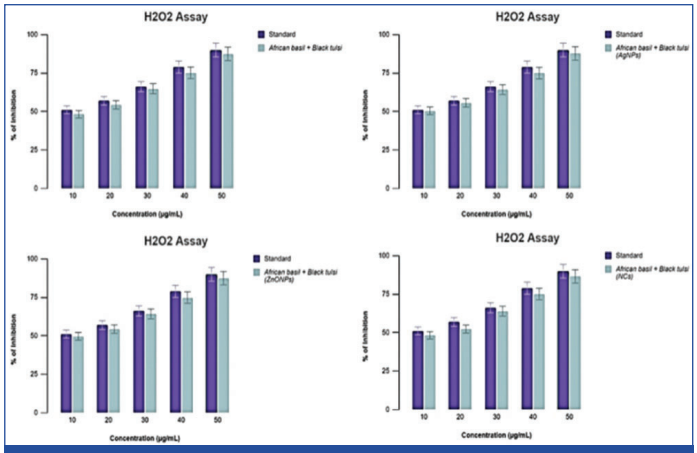
[Table/Fig-1]: Comparative antioxidant activity of *Ocimum tenuiflorum* and Ocimum grattissimum herbal formulation and its mediated nanoparticles, nanocomposites evaluated by using DPPH assay (The DPPH assay graph represents the percentage inhibition of DPPH radicals by different test groups at varying concentrations (10-50 µg/mL).

Incorporation of AgNPs and ZnONPs into the herbal formulation led to a significant enhancement in antioxidant activity, particularly evident at higher concentrations (40 and 50 µg/mL). The nanocomposite exhibited the highest antioxidant activity, surpassing both the herbal formulation alone and ascorbic acid.

H_2O_2 assay

The H_2O_2 assay graph presents the percentage inhibition of hydrogen peroxide by various test groups at concentrations ranging from 10 to 50 µg/mL. Ascorbic acid is marked as the standard in this graph. The test groups include the Ocimum-based herbal formulation, AgNPs, ZnONPs, and the nanocomposite. The graph demonstrates that all samples exhibit scavenging activity against H_2O_2, with AgNPs showing the highest inhibition (87.7) at 50 µg/mL, followed by ZnONPs (87.4) and the nanocomposite (86.5). These values are slightly lower than the standard ascorbic acid (89.9), but they highlight the potent antioxidant capabilities of the nanoparticles and nanocomposites, particularly at higher concentrations. Subtle changes may result from small effect sizes, short observation periods, or limited sample sizes.

Across all concentrations of H_2O_2 tested, the African basil + black tulsi formulations consistently exhibited significant antioxidant activity compared to the standard antioxidant (ascorbic acid). The African basil + black tulsi formulation demonstrated percentage inhibition values ranging from 48.2 to 87.4 against H_2O_2. At the highest concentration (50 µg/mL) of H_2O_2, the inhibition was 87.4 for the formulation compared to 89.9 for the standard. The formulation mediated AgNPs exhibited percentage inhibition values ranging from 50.4 to 87.7 against H_2O_2. At 50 µg/mL H_2O_2, the inhibition was 87.7 for the AgNPs compared to 89.9 for the standard. The ZnONPs showed percentage inhibition values ranging from 49.6 to 87.4 against H_2O_2. At 50 µg/mL H_2O_2, the inhibition was 87.4 for the ZnONPs compared to 89.9 for the standard. The nanocomposite demonstrated percentage inhibition values ranging from 48.2 to 86.5 against H_2O_2. At 50 µg/mL H_2O_2 the inhibition was 86.5 for the nanocomposite compared to 89.9 for the standard [Table/Fig-2].



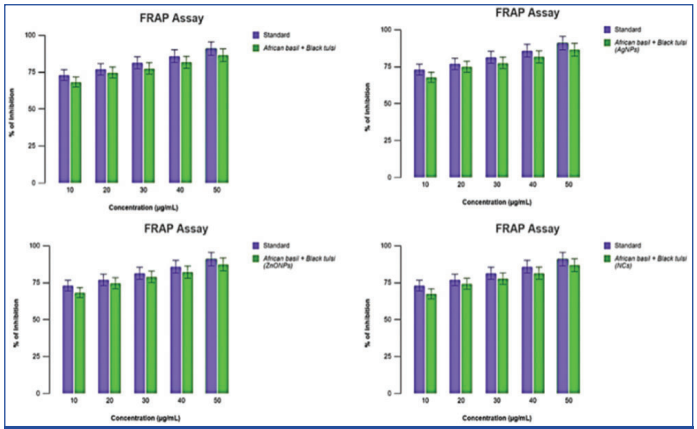
[Table/Fig-2]: Comparative antioxidant activity of *Ocimum tenuiflorum* and Ocimum grattissimum herbal formulation and its mediated nanoparticles, nanocomposites evaluated by using H_2O_2 assay.

Therefore, the African basil + black tulsi formulations, whether used alone or in the synthesis of nanoparticles (AgNPs, ZnONPs), and nanocomposite displayed notable antioxidant activities against hydrogen peroxide. While the formulations generally exhibited slightly lower inhibition percentages compared to the standard antioxidant (ascorbic acid), they demonstrated substantial scavenging capabilities across the concentration range tested.

FRAP assay

The FRAP assay graph illustrates the FRAP values of the test groups across concentrations (10-50 µg/mL). The standard in this graph is ascorbic acid, which serves as a benchmark for antioxidant capacity. The test groups compared include the Ocimum-based herbal formulation, AgNPs, ZnONPs, and the nanocomposite. The graph shows that all formulations exhibit strong reducing potential with ZnONPs achieving the highest FRAP value (87.38) at 50 µg/mL, followed closely by the nanocomposite (86.81). These values are slightly below that of ascorbic acid (90.89), reflecting the robust reducing abilities of the nanoparticles and their composites in neutralising oxidative stress. Subtle changes may result from small effect sizes, short observation periods, or limited sample sizes.

The FRAP of different formulations derived from African basil and black tulsi, including those synthesised with nanoparticles (AgNPs, ZnONPs) and a mediated nanocomposite, was evaluated and compared with a standard antioxidant using the FRAP assay. The results are presented as FRAP values at various concentrations (10, 20, 30, 40, 50 µg/mL) [Table/Fig-3].



[Table/Fig-3]: Comparative antioxidant activity of *Ocimum tenuiflorum* and Ocimum grattissimum herbal formulation and its mediated nanoparticles, nanocomposites evaluated by using FRAP assay.

The FRAP values of the formulations relative to the standard antioxidant (ascorbic acid) was tested across different concentrations. The African basil + black tulsi formulation exhibited FRAP values ranging from 68.32 to 86.45. At the highest concentration (50 µg/

mL), the FRAP value was 86.45 for the formulation compared to 90.89 for the standard. The AgNPs showed FRAP values ranging from 67.72 to 86.48. At 50 µg/mL, the FRAP value was 86.48 for the AgNPs formulation compared to 90.89 for the standard. The ZnONPs exhibited FRAP values ranging from 68.32 to 87.38. At 50 µg/mL, the FRAP value was 87.38 for the ZnONPs formulation compared to 90.89 for the standard.

The nanocomposite displayed FRAP values ranging from 67.43 to 86.81. At 50 µg/mL, the FRAP value was 86.81 for the nanocomposite compared to 90.89 for the standard [Table/Fig-3].

DISCUSSION

The findings of the present study highlight the potent antioxidant properties of an herbal formulation derived from *Ocimum tenuiflorum* and *Ocimum gratissimum*, particularly when incorporated into nanoparticle-based formulations. Green synthesis is an environmentally responsible method of producing biocompatible nanoparticles that can be used in biomedical applications by using natural and sustainable resources [13]. The herbal formulation demonstrated significant free radical scavenging activities across multiple assays, including DPPH, H_2O_2, and FRAP. The results consistently showed that the incorporation of AgNPs, ZnONPs, and nanocomposites significantly enhanced antioxidant activity, likely due to the synergistic interaction between the bioactive compounds in the extracts and the unique physicochemical properties of nanoparticles. AgNPs have anti-inflammatory qualities due to their ability to neutralize free radicals and inhibit inflammatory signaling pathways [14]. The investigation of nanoparticles for the treatment of different infections, cancer, inflammation, and other conditions linked to oxidative stress has significantly increased [15].

The DPPH assay results revealed a maximum inhibition of 90.91 at 50 µg/mL for both the pure herbal formulation and the nanocomposite. Notably, the nanocomposite exhibited enhanced activity at lower concentrations compared to the pure formulation, demonstrating the effectiveness of nanoparticle integration. Similar studies, such as those by Singh P et al., have shown that green-synthesised nanoparticles exhibit superior radical scavenging activities due to their high surface area and ability to interact efficiently with ROS [8].

In the H_2O_2 assay, the herbal formulation and its derivatives demonstrated robust scavenging capabilities, with AgNPs showing the highest inhibition (87.7) at 50 µg/mL, slightly below that of ascorbic acid (89.9). These results align with those reported by Varghese RM et al., where *Ocimum*-based ZnONPs and AgNPs exhibited significant hydrogen peroxide scavenging activity. The enhanced activity of nanoparticles is attributed to their ability to facilitate redox reactions more efficiently than bulk materials, highlighting the role of nanoparticle properties, such as size and surface charge, in determining antioxidant efficacy [5].

The FRAP assay results demonstrated the ferric reducing capacities of the formulations, with ZnONPs achieving the highest activity (87.38) at 50 µg/mL. While the FRAP values were slightly lower than ascorbic acid (90.89), they remained substantial and comparable to results from other green-synthesised nanoparticle studies [15,16]. The enhancement observed in ZnONPs may be due to their inherent catalytic properties and interactions with the herbal bioactive compounds, as reported in similar investigations involving other plant-derived nanoparticles [17].

Among the tested variables, nanoparticle incorporation consistently emerged as a significant factor in enhancing antioxidant efficacy. The ZnONPs exhibited superior performance in the FRAP assay, while the nanocomposite balanced its performance across all assays, showcasing its potential as a versatile antioxidant agent. This synergistic enhancement between herbal extracts and nanoparticles has also been documented in studies exploring the antioxidant potential of *Silybum marianum* and *Curcuma longa* formulations [18]. Optimisation of synthesis parameters, such as nanoparticle size and extract concentration, could further enhance efficacy and stability. These efforts would build upon the foundation laid by prior research and advance the development of novel antioxidant therapies [18-20].

The findings of this study have significant clinical implications, particularly in the development of natural and sustainable antioxidant therapies. The enhanced antioxidant activity observed in nanoparticle-based formulations, such as AgNPs, ZnONPs, and nanocomposites, demonstrates their potential as effective supplements or alternatives to traditional antioxidants [21]. These formulations can be incorporated into oral care products, such as mouthwashes or dental materials, to combat oxidative stress, prevent periodontal diseases, and promote oral health. Additionally, their biocompatibility and eco-friendly synthesis suggest they could be utilised in broader biomedical applications, such as wound healing, anti-inflammatory treatments, and cancer therapy. The synergy between herbal extracts and nanoparticles highlights an innovative approach to addressing oxidative damage in clinical settings.

Limitation(s)

While the present study provides valuable insights into the antioxidant properties of *Ocimum*-based formulations and their nanoparticle derivatives, certain limitations were encountered. First, the study primarily focused on in-vitro assays, which may not fully replicate in vivo conditions. The lack of cytotoxicity and biocompatibility assessments limits the understanding of the safety profile of these formulations for clinical applications. Additionally, variability in nanoparticle size and stability during the synthesis process may affect reproducibility and efficacy. The herbal extracts used were not subjected to detailed phytochemical analysis, making it difficult to identify the specific bioactive compounds responsible for the observed effects. Furthermore, the scalability of the green synthesis method for commercial production and long-term stability of the formulations under different storage conditions were not addressed. These limitations need to be addressed in future studies to validate the clinical relevance of the findings.

CONCLUSION(S)

The study provides compelling evidence of the potent antioxidant properties of an herbal formulation derived from African basil and black tulsi, particularly when incorporated into nanoparticle-based formulations. The herbal formulation consistently exhibited activity across multiple assays, surpassing the performance of the commonly used standard antioxidant, ascorbic acid. Incorporation of the herbal formulation into nanoparticle and nanocomposite structures further enhanced antioxidant activity, with the nanocomposite showing the highest antioxidant activity among all samples tested. This suggests that herbal-nanoparticle combinations could serve as effective alternatives or supplements to traditional antioxidants, offering a natural and sustainable approach to combating oxidative stress. The synergistic effects observed between the herbal extracts and nanoparticles highlight the potential for developing advanced antioxidant products with enhanced efficacy. Overall, the study underscores the promising prospects of herbal-nanoparticle combinations in the field of antioxidant research and product development.

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