

Research Article

Green Chemistry-Based Extraction, Phytochemical Screening, and Evaluation of Anti-Inflammatory Activity of *Aerva lanata*



Malath Saeed Alshahrani^{1*}, Wajan Khalid Alshahrani¹, Sara Abdullah A Assiri¹,
Waad Oudah Alasmari¹, Arwa Ali Alqahtani¹

Department of Pharmaceutics, College of Pharmacy, King Khalid University, Abha 61421, Saudi Arabia.

*Corresponding author: Malathsaed99@gmail.com



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Abstract:

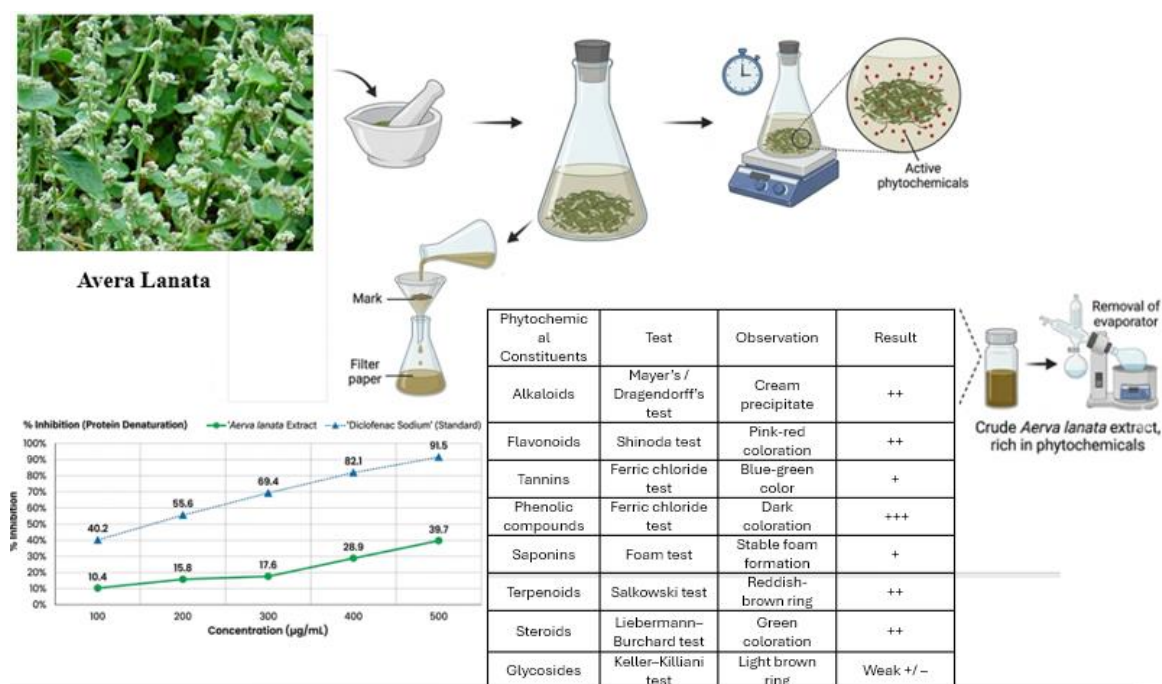
Green chemistry has emerged as an important approach in natural product research because it promotes environmentally friendly extraction techniques and minimizes the use of hazardous solvents. The present study investigated the green chemistry-based extraction, phytochemical screening, (PS) and evaluation of the anti-inflammatory activity of *Aerva lanata*, a medicinal plant widely used in traditional medicine. The plant material was collected from the Buldhana region of Maharashtra, India, shade-dried, powdered, and subjected to hydroalcoholic extraction using the maceration method to obtain the crude extract. Preliminary PS was performed using standard qualitative tests, which revealed the presence of several important phytoconstituents including alkaloids, flavonoids, phenolic compounds, tannins, saponins, terpenoids, and steroids, while glycosides were present in minor amounts. These phytochemicals are known for their significant pharmacological activities, particularly antioxidant and anti-inflammatory effects. The anti-inflammatory potential of the extract was evaluated using an in vitro protein denaturation inhibition assay at concentrations of 100–500 µg/mL. The results demonstrated a concentration-dependent increase in inhibition, with values of 10.4%, 15.8%, 17.6%, 28.9%, and 39.7% at concentrations of 100, 200, 300, 400, and 500 µg/mL, respectively. In comparison, the standard drug diclofenac sodium exhibited inhibition values of 40.2%, 55.6%, 69.4%, 82.1%, and 91.5% at the same concentrations. These findings indicate that *Aerva lanata* possesses notable AI, which may be attributed to the synergistic effects of its phytochemical constituents. Overall, the study supports the traditional medicinal use of *Aerva lanata* and highlights its potential as a promising natural source for developing safer anti-inflammatory therapeutic agents

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Graphical Abstract



1. Introduction

Green chemistry has emerged as an essential approach in modern scientific research, emphasizing environmentally friendly processes, reduced chemical waste, and the use of safer solvents and sustainable methodologies. (1,2). In natural product research and phytopharmaceutical development, green chemistry principles are crucial for designing extraction techniques that minimize environmental impact while maintaining the integrity and bioactivity of plant-derived compounds (3). Traditional extraction techniques frequently depend on substantial quantities of organic solvents, prolonged extraction times, and energy-intensive procedures, which may lead to environmental pollution and degradation of thermolabile phytochemicals. Therefore, the adoption of green chemistry-based extraction techniques offers a sustainable alternative that enhances the efficiency, selectivity, and safety of phytochemical investigations (4,5).

Medicinal plants have long served as an important source of therapeutic agents, providing numerous bioactive compounds used in traditional and modern medicine (6). Among these, *Aerva lanata* (family: Amaranthaceae), commonly known as mountain knotgrass, is a widely distributed medicinal herb located in tropical and subtropical areas, encompassing India, Sri Lanka, and other parts of Asia and Africa (7). The plant has been extensively employed in conventional Ayurvedic and folk pharmaceuticals for the management of diverse ailments such as kidney stones, urinary disorders, inflammation, diabetes, and respiratory conditions (8). Different components of the plant, encompassing leaves, stems, and roots, have been reported to possess diverse pharmacological properties such as diuretic, antimicrobial, antioxidant, hepatoprotective, and anti-inflammatory activities (9,10). The therapeutic potential of *Aerva lanata* is largely attributed to its rich phytochemical composition. Previous phytochemical studies have disclosed the existence of several classes of bioactive components, comprising flavonoids, alkaloids, phenolic compounds, tannins, saponins, and terpenoids (11,12). These phytoconstituents are known to exhibit significant biological activities, particularly anti-inflammatory effects, which play an important role in the management of inflammatory disorders. Inflammation is a complex biological response of the immune system triggered by infection, injury, or exposure to harmful stimuli. Although inflammation is a protective mechanism, prolonged or uncontrolled inflammatory responses can lead to chronic diseases such as arthritis, cardiovascular disorders, and metabolic syndromes. Therefore, the search for natural anti-inflammatory agents with fewer side effects has gained considerable attention in pharmaceutical and biomedical research (13,14).

Green chemistry-based extraction methods can significantly improve the recovery of phytochemicals while reducing the use of toxic solvents (15). Techniques employing eco-friendly solvents, optimized extraction parameters, and energy-efficient processes can enhance the yield and quality of plant extract. These approaches align with the principles of sustainable chemistry and support the development of safer herbal formulations and natural therapeutics. In this context, the present study focuses on the green chemistry-based extraction of *Aerva lanata*, followed by the extraction of PS and evaluation of its anti-inflammatory activity. This study aimed to identify the major phytochemical constituents of the plant extract and assess its potential as a natural anti-inflammatory agent. This study expands the research on sustainable extraction techniques and highlights the pharmacological significance of *Aerva lanata* as a promising medicinal plant for future drug development.

However, a clear research gap exists, as most studies on *Aerva lanata* rely on conventional extraction methods, with limited focus on green extraction approaches and their impact on phytochemical composition and anti-inflammatory activity. Additionally, integrated studies combining eco-friendly extraction, phytochemical screening, and standardized in vitro anti-inflammatory evaluation are scarce.

In this context, the present study investigated the green chemistry-based hydroalcoholic extraction of *Aerva lanata*, followed by phytochemical screening and evaluation of its anti-inflammatory activity. The novelty of this study lies in the integration of a sustainable extraction approach with systematic phytochemical analysis and concentration-dependent anti-inflammatory assessment using a protein denaturation assay, providing a reproducible and eco-friendly framework for phytopharmaceutical development. This study contributes to sustainable extraction research and highlights the therapeutic potential of *Aerva lanata* for future drug development.

Based on the above background and identified research gap, the present study was designed to perform green chemistry-based extraction of *Aerva lanata*, followed by phytochemical screening and evaluation of its anti-inflammatory activity using a protein denaturation assay.

2. Materials and Methods

2.1 Collection of Plant Material

The botanical substance *Aerva lanata* was collected from a nearby region of the Buldhana district, Maharashtra, India. A collection was conducted within the appropriate growing season to guarantee the availability of robust and developed plant specimens (Figure 1). Fresh aerial parts of the plant were carefully harvested from natural habitats, ensuring that the samples were free of disease, insect damage, and environmental contamination. After collection, the plant matter was thoroughly cleaned to remove adhering soil, dust, and other extraneous matter. The gathered samples were transported to the laboratory in clean polyethylene bags to prevent contamination and deterioration. The plant specimens were then shade-dried at room temperature under adequate ventilation for several days until a constant weight was obtained. Shade drying was preferred to prevent the degradation of thermolabile phytoconstituents and preserve the phytochemical integrity of the plant material. Once completely dried, the plant material was coarsely powdered using a mechanical grinder and stored in airtight containers at room temperature until further use for extraction and phytochemical analysis. Proper labeling and documentation of the collected plant material were maintained throughout the process to ensure the traceability and reproducibility of the study.



Figure 1. *Aerva lanata* plant (mountain knotgrass) collected from Buldhana district, Maharashtra, India. The image shows the aerial parts used for extraction, ensuring authenticity and proper identification of the plant material.

2.2 Maceration Extraction

The dried plant material of *Aerva lanata* was subjected to maceration extraction to obtain a crude plant extract (Figure 2). Initially, the shade-dried plant material was coarsely powdered using a mechanical grinder to increase the surface area for optimal extraction of the phytochemicals. The powdered botanical substance was accurately weighed and transferred to a clean, dry conical flask. A suitable quantity of hydroalcoholic solvent (ethanol–water mixture) was added to the powdered plant material at a ratio sufficient to completely immerse the sample. The mixture was then closed or kept at room temperature for maceration. The flask was occasionally shaken and stirred at regular intervals to facilitate solvent penetration and enhance the extraction of phytoconstituents from the plant matrix. The maceration process was continued for approximately 48–72 h to ensure the maximum extraction of bioactive compounds. After completion During the extraction time, the combination was filtered through a muslin cloth and subsequently through Whatman No. 1 filter paper to eliminate plant debris and acquire a clear filtrate (16).

The obtained filtrate was then concentrated by solvent evaporation under reduced pressure using a spinning evaporator or by gentle evaporation on a water bath at a controlled temperature. The concentrated extract was dried to obtain a semi-solid crude extract. The dried extract was weighed to ascertain the % yield and stored in an airtight container at 4 °C until further use for PS and evaluation of AI.

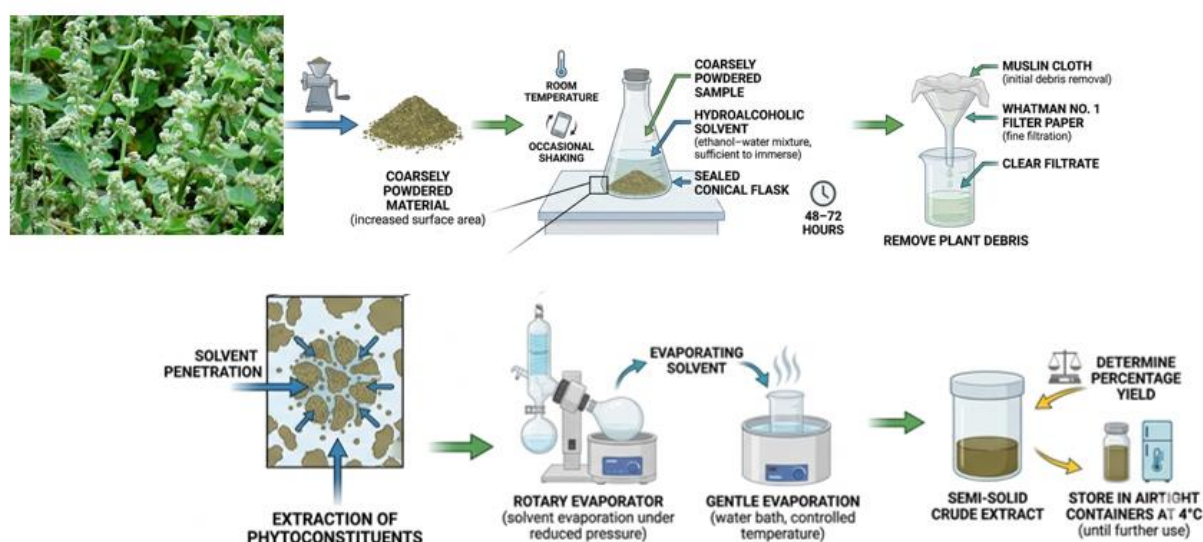


Figure 2. Hydroalcoholic maceration extraction process of *Aerva lanata*. The setup illustrates soaking of powdered plant material in ethanol–water mixture for efficient extraction of phytoconstituents.

2.3 Phytochemical Screening (PS)

Preliminary PS of the *Aerva lanata* extract was performed to identify the presence of major classes of secondary metabolites, such as alkaloids, flavonoids, tannins, saponins, phenolic compounds, glycosides, terpenoids, and steroids. Standard qualitative chemical tests were performed according to the established PS procedures. For analysis, a small quantity of crude extract obtained from the maceration process was dissolved in a suitable solvent to prepare the test solution. The prepared extract was then subjected to different qualitative tests to ascertain the presence of particular phytochemical constituents (17).

The presence of alkaloids was determined using Mayer's and Dragendorff's reagents, resulting in the formation of a cream or reddish-brown precipitate, indicating a positive result. Flavonoids were identified using the Shinoda test, where the development of a pink or red coloration after the addition of magnesium turnings and concentrated hydrochloric acid indicated the presence of flavonoids. Tannins and phenolic compounds were detected using the ferric chloride test, where the appearance of a dark blue or greenish-black color confirmed their presence. Saponins were detected using the foam test, where the establishment of a steady froth upon vigorous shaking of the extract with water indicated the presence of saponins. Terpenoids were identified using the Salkowski test, where the formation of a reddish-brown coloration at the interface after the addition of concentrated sulfuric acid indicated the presence of terpenoids. The presence of steroids was evaluated using the Liebermann-Burchard reaction, which produces a green or bluish color in the presence of steroidal compounds (18).

All tests were performed in triplicate to ensure the reliability of the results. The observations obtained from these qualitative tests provided preliminary information about the phytochemical constituents present in the *Aerva lanata* extract, which may contribute to its biological and pharmacological properties.

2. 4 Evaluation of Anti-inflammatory Activity

The anti-inflammatory activity of *Aerva lanata* extract was assessed using the in vitro protein denaturation inhibition method, which is a widely used model for screening potential anti-inflammatory agents. The underlying premise of this method is based on the capacity of certain compounds to inhibit heat-induced protein denaturation. Protein denaturation is a well-documented cause of inflammation, and substances that prevent protein denaturation may possess significant anti-inflammatory properties (15).

The reaction mixture consisted of 1% aqueous solution of bovine serum albumin (BSA) and various concentrations of the *Aerva lanata* extract prepared in an appropriate solvent. Briefly, 1 mL of plant extract at diverse concentrations (for example 100, 200, 300, 400, and 500 µg/mL) was mixed with 1 mL of 1% BSA solution in test tubes. The pH of the mixture was adjusted to approximately 6.3 utilizing a low amount of 1N hydrochloric acid if necessary. The reaction mixtures were incubated at ambient temperature for 10 minutes. After incubation, the test tubes were subjected to heating in a water bath at 55–60 °C for approximately 20 minutes to induce protein denaturation. Subsequent to heating, the samples were let to cool to ambient temperature. The turbidity of the reaction mixture was calculated spectrophotometrically at 660 nm using a UV-visible spectrophotometer (19). A control sample containing BSA solution without plant extract was prepared under the same conditions. A standard anti-inflammatory drug (such as diclofenac sodium or aspirin) used as a reference point for comparison. All experiments were conducted in duplicate to guarantee accuracy. The reproducibility of the outcomes.

3. Results and Discussion

3.1 Photochemical Screening

Preliminary PS of the hydroalcoholic extract of *Aerva lanata* revealed the presence of multiple important classes of secondary metabolites (Figure 3). Qualitative tests indicated the presence of alkaloids, flavonoids, phenolic compounds, tannins, saponins, terpenoids, and steroids, while some constituents, such as glycosides,

were moderately present depending on the solvent system used. These phytochemicals are recognized for their contribution to the pharmacological activity of medicinal plants, including antioxidant and anti-inflammatory properties.

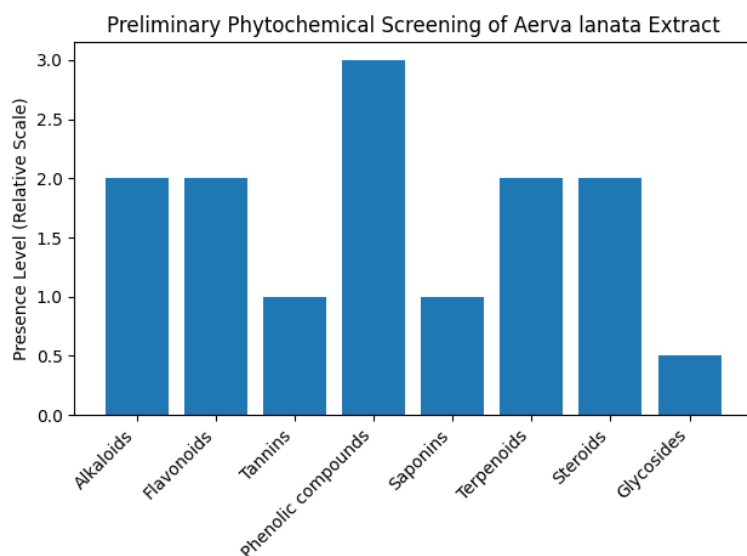


Figure 3. Preliminary phytochemical screening of *Aerva lanata* extract. Representative qualitative tests indicating the presence of major secondary metabolites such as flavonoids, alkaloids, and phenolics.

The detection of flavonoids and phenolic compounds in the extract is particularly significant because these compounds are known to exhibit potent antioxidant activities that neutralize free radicals and diminish oxidative stress. Flavonoids such as quercetin, kaempferol, and isorhamnetin have been identified in *Aerva lanata*, contributing to its pharmacological potential. Similarly, the alkaloids and saponins present in the plant may contribute to its anti-inflammatory and antimicrobial effects. Studies have reported that *Aerva lanata* contains several bioactive compounds, including β -sitosterol, α -amyrin, tannins, and steroids, which are associated with various pharmacological properties. The presence of multiple classes of phytochemicals suggests that the biological activities of *Aerva lanata* may result from synergistic interactions between these compounds. Many of these phytoconstituents inhibit inflammatory mediators, reduce oxidative stress, and protect cells from damage, thereby supporting the conventional medicinal use of this species. In addition, the phytochemical richness of *Aerva lanata* makes it a promising candidate for the development of herbal formulations and natural therapeutic agents.

3.2 Evaluation of Anti-inflammatory activity

The AI of the *Aerva lanata* extract was assessed using the protein denaturation inhibition assay, which is widely utilized as an in vitro model to evaluate anti-inflammatory potential (Table 1) and (Figure 4). Protein denaturation is a well-documented cause of inflammation, and compounds capable of inhibiting this process may possess significant anti-inflammatory properties. The hydroalcoholic extract of *Aerva lanata* showed concentration-dependent inhibition of protein denaturation. As the concentration of the extract increased from 100 to 500 $\mu\text{g/mL}$, the percentage inhibition also increased significantly. The highest inhibition was observed at 500 $\mu\text{g/mL}$, indicating strong anti-inflammatory activity. The activity of the plant sample was compared with the standard anti-inflammatory drug diclofenac sodium.

The results of the present study are consistent with those of previous studies. Several studies have reported that *Aerva lanata* possesses significant anti-inflammatory activity owing to the presence of flavonoids, phenolic compounds, alkaloids, and terpenoids, which are known to inhibit inflammatory mediators and reduce protein denaturation. These phytoconstituents also contribute to antioxidant activity, which further

supports their anti-inflammatory effects. Previous pharmacological studies have demonstrated that extracts of *Aerva lanata* extracts significantly inhibit inflammatory responses and reduce oxidative stress markers.

The anti-inflammatory mechanism of the extract may be attributed to multiple pathways associated with its phytochemical constituents. Flavonoids and phenolic compounds inhibit cyclooxygenase (COX) and lipoxygenase (LOX) enzymes, thereby reducing the synthesis of pro-inflammatory mediators, such as prostaglandins and leukotrienes. Additionally, these compounds exhibit strong antioxidant activity, which helps scavenge reactive oxygen species and minimize oxidative stress-induced inflammation. Alkaloids and terpenoids further suppress the release of inflammatory cytokines and stabilize cellular membranes. Moreover, the ability of these phytoconstituents to prevent protein denaturation directly supports the observed in vitro anti-inflammatory activity. This overall effect is likely due to the synergistic interaction of these bioactive compounds, which enhances the therapeutic potential of the extract. Overall, these findings suggest that *Aerva lanata* possesses promising anti-inflammatory potential and may serve as a natural source of therapeutic agents for the management of inflammatory disorders.

Table 1. Anti-inflammatory activity of *Aerva lanata* Extract by Protein Denaturation Assay

Concentration ($\mu\text{g/mL}$)	% Inhibition (<i>Aerva lanata</i> Extract)	% Inhibition (Diclofenac Sodium)
100	10.4	40.2
200	15.8	55.6
300	17.6	69.4
400	28.9	82.1
500	39.7	91.5

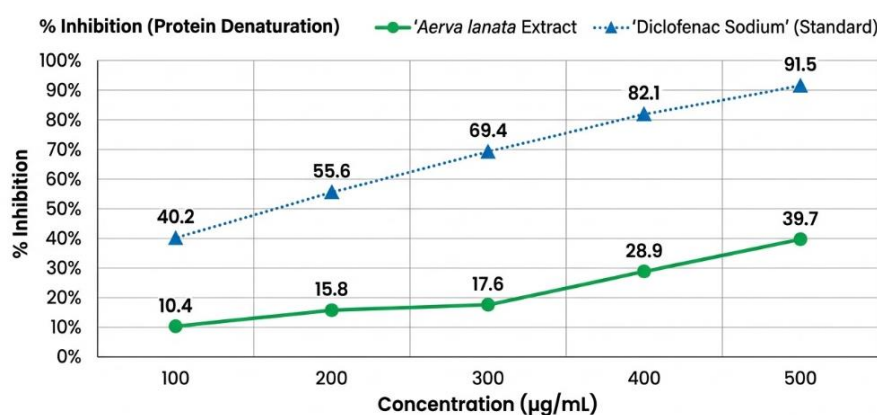


Figure 4. In vitro anti-inflammatory activity of *Aerva lanata* extract using protein denaturation assay. graph showing concentration-dependent percentage inhibition (100–500 $\mu\text{g/mL}$) compared with standard diclofenac sodium.

4. Conclusion

The present study demonstrates the effective application of green chemistry principles for the extraction and evaluation of bioactive constituents from *Aerva lanata*. Hydroalcoholic maceration is a sustainable and efficient method for obtaining phytochemicals while minimizing the use of harmful solvents. Preliminary phytochemical screening confirmed the presence of major secondary metabolites, including flavonoids, alkaloids, phenolic compounds, tannins, saponins, terpenoids, and steroids, which are known to contribute to therapeutic activity. The in vitro anti-inflammatory activity, assessed using a protein denaturation inhibition assay, revealed a clear concentration-dependent response, indicating the potential of the extract to inhibit inflammatory processes. Although the activity was lower than that of diclofenac sodium, the results support the traditional medicinal use of the plant and highlight its potential as a natural anti-inflammatory agent. Future research should focus on the isolation and characterization of active compounds, detailed mechanistic studies,

and in vivo evaluations to validate their efficacy and safety, thereby supporting the development of eco-friendly phytopharmaceutical formulations.

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None

Conflict of Interest

The authors declare no conflict of interest.

Authors' Contribution

Authors contribution includes "Conceptualization, and methodology, software, validation, and formal analysis investigation, resources, data curation, writing—original draft preparation, writing—review and editing, visualization, supervision, project administration, Malath Saeed Alshahrani, Wajan Khalid Alshahrani, Sara Abdullah A Assiri, Waad Oudah Alasmari, and Arwa ali Alqahtani acquisition. All authors have read and agreed to the published version of the manuscript.

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