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**Research Article**

# To Study of *Artemisia nilagirica* Leaves for their Antithyroid, Oxidative and Antihyperglycemic Properties

**Jayshri Hiradas Bairagi[1], Gurnani Haritha[2], Latika Yadav[3], Swapnil Garg[4], Varsha Rani[5], Sowjanya Pulipati[6], Rajeev Ramachandra Kolgi[7], Richa Pundir[8] and Sharangouda J. Patil[9]***

[1]Oyster Institute of Pharmacy, Gut no 613, Nagonachi Wadi, Golatgaon, Near Karmad, Sambhajinagar- 431201, Maharashtra, India
[2]K. L. College of Pharmacy, Koneru Lakshmaiah Education Foundation, Vaddeswaram-522302, Andhra Pradesh, India
[3]Department of Home Science, Vijay Singh Pathik Government Post Graduate Degree College, Kairana, Shamli - 247774, (Affiliated to Maa Shakumbhari University, Punwarka, Saharanpur), Uttar Pradesh India
[4]Ras Bihari Bose Subharti University, Nanda ki chowki, Prem Nagar, Dehradun - 248007, Uttarkhand, India
[5]Department of Pharmacology, Himalayan Institute of Pharmacy and Research, Raja Wala Road Selaqui, (Affiliated to Uttarakhand Technical University, Dehradun) Dehradun 248007, Uttarakhand, India
[6]Vignan Pharmacy College, Vadlamudi, Guntur - 522213, Andhra Pradesh, India
[7]Department of Chemistry and Biochemistry, Government Science College, Bengaluru-560001, Karnataka, India
[8]Ras Bihari Bose Subharti University, Nanda Ki Chowki, Prem Nagar, Dehradun - 248007, Uttarakhand, India
[9]Department of Zoology, NMKRV College for Women, Bengaluru -560011, Karnataka, India

***Corresponding author: Sharangouda J. Patil,** Department of Zoology, NMKRV College for Women, Bengaluru - 560011, Karnataka, India, e-Mail: shajapatil@gmail.com

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**ABSTRACT**

**Background and Objective:** The tiny, fragrant shrub *Artemisia nilagirica* (Clarke) is a member of the Asteraceae family. This plant's leaves and flowering top have been used in traditional medicine as an antimalarial, nerve tonic, and anticancer drug, and there is evidence of anti-leishmanial efficacy. **Material and Methods:** The plant specimen was obtained from the Ooty district in Tamil Nadu, India, during the month of December. It was subsequently authenticated by NMKRV College for Women, Bengaluru, Karnataka, India. The plant specimens were stored in the herbarium of the NMKRV College for Women, Bengaluru, Karnataka, India. **Results:** The morphological, microscopic, organoleptic, and other WHO-recommended procedures for standardization were investigated in a powdered sample of *Artemisia nilagirica* (Clarke) leaf. Phytochemical investigation revealed the existence of numerous elements such as alkaloids, amino

acids, carbohydrates, flavonoids, glycosides, terpenoids, steroids, saponins, essential oils, tannins and phenol in diverse extracts. **Conclusion:** Pharmacognostic and phytochemical analysis of *Artemisia nilagirica* (Clarke) leaves would be useful for authenticating raw material or crude medication.

**Keywords:** Antithyroid, Antioxidant, Antihyperglycemic, *Artemisia nilagirica* leaves.



## INTRODUCTION

*Artemisia nilagirica* is commonly referred to as 'Nagdona' in the Hindi language. The plant in question is a perennial herbaceous species with aromatic properties, classified within the Asteraceae family. It typically reaches a height of 150 cm and thrives in hilly regions of India, specifically on soils rich in nitrogen [1, 2]. *Artemisia nilagirica* (Clarke) is distributed across several regions including Europe, Asia, northern Africa, Alaska, and North America, indicating its indigenous presence in these areas. The plant exhibits a stem that is angular in shape, with grooves and a reddish-purple hue. It produces small yellow flowers during the period from July to September. The leaves of the plant are pinnate, measuring between 5-20 cm in length. They possess a smooth texture and are characterized by a dark green coloration on the upper surface, while the under surface appears pale green and is adorned with small white hairs [3].

The Artemisia genus and its constituent species have been extensively researched due to their diverse range of biological activity. The medicinal properties of Artemisia annua L. have been shown to be notable due to the presence of artemisinin. *Artemisia mongolica* is a significant constituent of the genus, characterized by its abundance of lactone derivatives of Sesquiterpene and diverse pharmacological properties. The many species within the genus exhibited potent antibacterial and antifungal capabilities against pathogenic organisms affecting humans, livestock, and plants. The bioactive chemicals and extracts derived from several species of Artemisia have been found to possess antiproliferative and apoptotic properties. *Artemisia nilagirica* is found in the Western Ghats region of India and has been historically utilized by indigenous healers in the locality for the management of infectious ailments and the prevention of toxicity [4].

*Artemisia nilagirica* (Clarke) is commonly employed in traditional medicine to address a range of health conditions, including but not limited to, diminished appetite, indigestion, constipation, parasite infection, cramping, colds, fever, gout, nervousness, leprosy, pruritus, malaria, fungal infection, leishmaniasis, and cancer. Furthermore, this botanical specimen has been employed for its antibacterial, antioxidant, anthelmintic, and insect repellant properties. The medicinal benefits of *Artemisia nilagirica* (Clarke) are attributed to the presence of secondary metabolites, including flavonoids, terpenoids, saponins, polysaccharides, and essential oil constituents [5].

*Artemisia nilagirica* has been utilized in traditional medicine to address a range of health conditions, including but not limited to, diminished appetite, indigestion, constipation, motion sickness, parasite infections, irregular menstruation, menstrual pain, cramping, colds, epilepsy, typhoid, TB, urinary calculi, and fever. The objective of this study is to establish a connection between the conventional principles and applications of herbal medications, herbal products, and specific phytochemicals in order to explore their potential as phytomedicine, employing contemporary scientific methodologies [6].

The presence of coumarins, flavonoids, tannins, terpenoids, volatile oil, carbohydrates, and sterols in the leaves has been observed in phytochemical research. The primary objective of

this study is to examine the antithyroid, antioxidative and anti-hyperglycemic properties of *Artemisia nilagirica*.

## MATERIALS AND METHODS

The plant specimen was obtained from the Ooty district in Tamil Nadu, India, during the month of December. It was subsequently authenticated by Department of Botany, NMKRV College for Women, Bengaluru - 560011, Karnataka, India. The plant specimens were stored in the herbarium of the NMKRV College for Women, Bengaluru, Karnataka, India.

### Reagents and Chemicals

The chemicals and solvents utilized in this study were obtained from reputable suppliers, including Merck (Germany), SD Fine Chemical (India), Loba, Research Lab, and Ranchem (India).

### Morphology

The determination of leaf morphology involved the placement of leaves onto the stage of a basic microscope, followed by observation via a 6X lens.

### Organoleptic and Microscopic analysis

The leaves of *Artemisia nilagirica* (Clarke) in a healthy state were subjected to a cleaning process using deionized water. Subsequently, the leaves were dried under shade and subsequently crushed to obtain a coarse powder. The powder was stored in a hermetically sealed bottle of amber hue throughout the duration of the study. It was separated into two distinct halves. The initial portion of the cleaned leaves was conserved in a solution composed of Formaldehyde-Acetic acid-Alcohol (consisting of 5 ml of Formalin, 5 ml of Glacial acetic acid, and 90 ml of 70% Ethanol) in order to facilitate future utilization in anatomical investigations. The second portion of the cleaned leaves was subjected to shade drying, followed by grinding using a mechanical grinder. The resulting powder was then stored in an air-tight container for subsequent analysis utilizing powder microscopy and phytochemical tests. The powder was evaluated for its color, odor, texture, and taste by being placed on a Petri plate. The hue was determined by juxtaposing the powder against a white surface and examining it under natural lighting conditions. The olfactory and gustatory properties were assessed through the perception of a desiccated substance, as well as the immersion of a small quantity of the substance in heated water. Microscopic analysis was conducted using sections that were 10-12 µm thick, prepared similar to previously reported methods with minor adjustments. Photographic pictures were captured utilizing the Nikon Lab Photo 2 tiny instrument [7, 8].

### Phytochemical Analysis

A quantity of 50 grams of powdered *Artemisia nilagirica* (Clarke) leaves was subjected to extraction using several solvents, including petroleum ether, n-hexane, diethyl ether, benzene, ethylene dichloride, chloroform, and ethanol, in a sequential manner using a soxhlet device. The sample that was obtained was utilized for the purpose of conducting a phytochemical analysis. One gram of different extracts derived from *Artemisia nilagirica* (Clarke) leaves were dissolved in 100 milliliters of a suitable solvent, referred to as the mother solvent, individually to create a stock solution with a concentration of 1% weight/volume. These stock solutions were subsequently utilized for phytochemical screening through the application of the following chemical analysis methods [9, 10].

### Test for Alkaloids

#### Dragendorff's Test

Each stock solution of various extracts was treated with 1 ml of Dragendorff's reagent,

namely Potassium Bismuth Iodide Solution. The presence of alkaloids can be inferred when an orangered precipitate is formed.

**Mayer's Test**

A volume of 1 ml of Mayer's reagent, namely Potassium Mercuric Iodide Solution, was introduced to individual 1 ml stock solutions of different extracts. The presence of alkaloids can be inferred when a precipitate of white yellow or cream color is formed.

**Test for Glycosides**

**Keller-Killiani Test**

A 5 ml aliquot of a stock solution comprising different extracts was combined with a combination consisting of 5 ml of water and 2 ml of glacial acetic acid, which was supplemented with a single drop of ferric chloride solution. Subsequently, 1 ml of concentrated sulphuric acid was added to the mixture. The presence of glycosides can be inferred from the sequential production of a brown ring at the interface, followed by a violet ring beneath the brown ring, and the subsequent formation of a greenish ring in the acetic acid layer just above the brown ring, which eventually spreads across the layer [11, 12].

**Baljet Test**

A small quantity of picric acid was introduced into each 1 ml stock solution of different extracts individually. The observation of an orange coloration serves as an indication of the existence of glycosides.

**Test for Tannins and Phenol**

**Lead Acetate Test**

A volume of approximately 2 milliliters of a basic lead acetate solution was introduced into individual 2 milliliter aliquots of various extract stock solutions. The occurrence of a white precipitate signifies the existence of tannins.

**Ferric Chloride Test**

Each stock solution of varied extracts was individually supplemented with 1 ml of ferric chloride solution. The manifestation of a blue-black or brownish-green hue signifies the existence of tannins.

**Test for Amino Acids**

**Ninhydrin Test**

In each test tube, 3 drops of a 5% Ninhydrin solution were added to 3 ml of various extract stock solutions. The test tubes were then placed in a boiling water bath and heated for a duration of 10 minutes. The occurrence of a purple or bluish hue signifies the existence of amino acids.

**Test for Carbohydrates**

**Fehling's Test**

One milliliter of Fehling's solution A and B was separately added to each one milliliter stock solution of various extracts in a test tube. The mixture was then cooked in a boiling water bath. The observation of the formation of a brick red precipitate signifies the existence of sugar.

**In-vivo Antioxidant Activities**

**Lipid Peroxidation**

Lipid peroxidation refers to the process of oxidative degradation of lipids that possess many

carbon-carbon double bonds. Lipid peroxidation is a widely recognized process of cellular damage in both plant and animal organisms, serving as a reliable marker for assessing oxidative stress levels in cells and tissues. Lipid peroxides exhibit inherent instability, leading to their decomposition and subsequent formation of various chemicals, including reactive carbonyl compounds. Numerous hazardous substances are generated as a result of lipid peroxidation. These factors have an impact on locations that are distant from the area where they are produced. Therefore, they exhibit characteristics of harmful "second messengers". Lipid peroxidation poses a significant risk to membrane lipids. The integrity of cellular organelles, such as mitochondria, plasma membranes, endoplasmic reticulum, lysosomes, and peroxisomes, relies heavily on the structural integrity of the membrane. Consequently, any damage inflicted by the LP (lipid peroxidation) might have severe consequences for the cell's functionality and overall viability [12, 13].

**Glutathione Assay**

Glutathione is a tripeptide that exhibits a wide distribution in various plant and animal species. Glutathione functions as a nucleophile co-substrate in the detoxification process of xenobiotics via glutathione transferases. Additionally, it plays a crucial role as an electron donor to glutathione peroxidases, aiding in the reduction of hydro peroxides. Glutathione plays a crucial role in the transportation of amino acids and the preservation of the sulfhydryl reduction state of proteins. The concentration of glutathione varies from a few micromolar in plasma to several millimolar [14, 15].

**Antithyroid Activity**

Thyroid hormones are known to exert significant influence on various physiological processes, including but not limited to development, metabolism, thermoregulation, and growth. Nevertheless, in several pathological circumstances such as Graves' disease, thyroid tumors, and pituitary gland tumors, there is a stimulation of thyroid cells leading to an increased production of hormones, ultimately resulting in a state of hyperthyroidism. Changes in the concentration of these hormones not only result in modifications to the basal metabolic rate but also give rise to a multitude of health complications. Hyperthyroidism, when left untreated, can potentially lead to the development of prevalent health conditions such as diabetes mellitus and cardiovascular disorders. The induction of hyperthyroidism in rodents was successfully accomplished by administering continuous administration of l-thyroxine, a synthetic variant of thyroid hormone, which closely mimics the condition observed in humans. Hyperthyroidism is associated with the occurrence of hepatic oxidative damage, osteoporosis, cardiac failure, and an elevated susceptibility to myocardial infarction [16-18].

**Antihyperglycemic Activity**

The glucose oxidase method is commonly employed for the assessment of serum glucose levels. The process of glucose oxidation to gluconic acid is facilitated by the enzyme glucose oxidase. The hydrogen peroxide that has been produced is identified through the utilization of a chromogenic oxygen acceptor known as phenolaminophenazone, which is present alongside peroxidase [19, 20].

## RESULTS AND DISCUSSION

**Morphological evaluation**

The analysis of the morphological characteristics, including organoleptic and wide morphological parameters, reveals that the leaf arrangement of the specimen is opposite, exhibiting lobed, laciniate, or pinnatipartite features, with a length ranging from 5 to 14 cm. The blooms exhibit a high number of petals, characterized by their diminutive size, delicate

golden hue, and delightful fragrance. The fruits can be classified as achenes [21, 22].

**Microscopical Evaluation**

Microscopic evaluation plays a crucial role in enlarging the intricate features of small entities, thereby verifying the structural characteristics of plant-based drugs. This process provides corroborating evidence when paired with other analytical criteria, such as cell kinds and cell inclusion details [23, 24].

**Determination of Moisture Content**

It is necessary to ascertain the loss on drying of plant materials and exercise control over their water content. This is particularly significant for materials that exhibit high moisture absorption rates and are prone to rapid degradation in the presence of water. The examination assesses the presence of both water and volatile substances [25].

**Foreign Organic Matter**

It is imperative that drugs are devoid of any presence of molds, insects, animal excrement, and other contaminants, including soil elements, stone, and extraneous substances. The quantity of extraneous substances shall not exceed the specified percentage as outlined in the monograph [26].

**Determination of Volatile Oil**

The volatile oil content of *A. nilagiria* was determined using the Clevenger equipment, with fresh leaves being utilized for the analysis. The measurements were quantified in milliliters and recorded systematically [27].

**Ash Value**

The term "ash" refers to the residual substance that remains after the process of burning of the crude medicine. The residue often consists of naturally occurring inorganic salts that adhere to the medication. The variation is contingent upon the specific soil conditions within defined boundaries. Additionally, it may encompass the intentional inclusion of inorganic substances for the goal of adulteration. Therefore, the evaluation of ash value provides a foundation for assessing the authenticity and purity of a medicine, as well as indicating potential adulteration or contamination with organic substances. Consequently, ash values play a crucial role in evaluating the overall quality and purity of the drug [28].

**Extractive Values**

The extractive values were determined for various solvents, including methanol, ethanol, petroleum ether, aqueous solution, ethyl acetate, and chloroform. The aqueous extract is considered to have a better value due to its reduced risk of contamination, making it a favored choice over the methanol extract.

**Phytochemical screening of *Artemisia nilagirica***

The findings derived from the examination of phytochemical analysis of different extracts are displayed in a tabular format. The methanolic extract exhibited the presence of flavonoids, tannins, saponins, and coumarins. The provided extracts do not exhibit the presence of alkaloids, amino acids, and glycosides [28, 29].

**Table 1: Phytochemical Screening**

| Sr. No. | Test | Ethanol | Aqueous | Methanol | PET ether |
|---|---|---|---|---|---|
| 1 | Alkaloids | - | - | - | - |
| 2 | Amino acids | - | - | - | - |

| | | | | | |
|---|---|---|---|---|---|
| 3 | Carbohydrates | - | + | - | - |
| 4 | Flavonoids | + | + | + | + |
| 5 | Glycosides | - | - | - | - |
| 6 | Coumarins | + | + | + | + |
| 7 | Tannins | + | + | + | - |
| 8 | Saponins | + | + | + | - |
| 9 | Terpenoids | - | - | - | + |
| 10 | Volatile oil | - | + | - | + |
| 11 | Sterols | - | - | - | + |

**Estimation of Phenolic Content**

The modified Folinciocalteu technique was employed in the current investigation to quantify the total phenolic content in the extract. The values are quantified in terms of gallic acid equivalents and subsequently organized in a tabular format. The phenolic content present in the extract was determined using linear regression analysis. Phenolic chemicals are a class of secondary metabolites that arise from the pentose phosphate, shikimate, and phenylpropanoid pathways within the plant kingdom. Phenolics possess antioxidant characteristics due to their redox capabilities, enabling them to function as reducing agents, hydrogen donors, and singlet oxygen quenchers. Additionally, they possess metal chelation characteristics [29, 30].

**Estimation of Flavonoid Content**

Flavonoids are a class of polyphenolic chemicals that possess well-established characteristics, such as the ability to scavenge free radicals, inhibit hydrolytic and oxidative enzymes, and exert anti-inflammatory effects. There is evidence indicating a potential correlation between the biological effects of certain chemicals and their antioxidant properties. The methanolic extract was determined to have a total flavonoid concentration of 84.299 mg/g of extract. Flavonoids are recognized for their capacity to scavenge free radicals and exhibit antioxidant activities. Furthermore, the physiological features of certain flavonoids are influenced by the locations of their substituents. The flavonols that possess an ortho or para hydroxyl group in the 2-phenyl ring have been identified [30, 31].

**Estimation of Tannin Content**

The quantification of tannins was conducted using spectrophotometry following the oxidation of the sample with a Folindenis reagent under alkaline conditions. This methodology is founded upon the principles of redox reactions and the utilization of reducing substances inside the sample. The total tannin content in the methanolic extract of *Artemisia nilagirica* leaves was determined to be 757.50 ± 3.8420 mg of tannic acid equivalent per gram of extract [32, 33].

**Antioxidant activity**

The observed outcomes for the methanolic extract of *A. nilagirica* can be ascribed to various factors, such as the hindrance of ferry-per ferryl complex formation, the elimination of OH- or superoxide radical through scavenging, or the alteration of the Fe+++ to Fe++ ratio, thereby reducing the rate of conversion from ferrous to ferric state via chelation of the ion itself. The $IC_{50}$ value for the standard ascorbic acid was determined to be 15.10 mg/ml, while the $IC_{50}$ value for the methanolic extract of *A. nilagirica* was found to be 40.14 mg/ml [32, 33].

**Table 2: Antioxidant Activity of Methanolic Extract of *A. nilagirica***

| Sr. No. | Concentration (µg/ml) | Inhibition of Ascorbic acid (%) | Inhibition of *A. nilagirica* (%) |
|---|---|---|---|
| 1 | 10 | 52.01±0.345 | 57.24±0.47 |
| 2 | 20 | 55.2±0.234 | 38.02±0.24 |
| 3 | 30 | 60.47±0.014 | 49.35±0.784 |
| 4 | 40 | 66.14±0.89 | 59.35±0.987 |
| 5 | 50 | 71.18±0.078 | 58.35±0.78 |
| | **$IC_{50}$** | **15.10 mg/ml** | **40.14 mg/ml** |

**Antithyroid Activity**

Thyroid hormones, namely triiodothyronine (T3) and thyroxine (T4), play a crucial role in the control of various physiological processes within the body. These processes encompass lipid and glucose metabolism, oxygen consumption, as well as key functions like development, reproduction, and growth. Deviation from their typical concentrations can lead to various biochemical and clinical irregularities, including hypothyroidism and hyperthyroidism. Prolonged administration of exogenous LT4 therapy has the potential to modify thyroid function by impeding the production of thyroid hormones, hence causing disturbance to the thyroid axis and giving rise to various disorders. Hyperthyroidism can be succinctly described as a condition characterized by elevated levels of blood T3 and T4, accompanied by a reduction in serum TSH, a pituitary hormone responsible for regulating thyroid function. The current investigation observed that the administration of mean extracts effectively prevented the dose-dependent increases in blood T3 and T4 levels generated by LT4, while also causing a significant drop in serum TSH concentrations [31-33].

**Table 3: Serum thyroid hormone levels in the $lt_4$ and test materials treated rats**

| Groups | Thyroid stimulating hormone(µg/ml)TSH | Triiodothyronine (µg/ml) $T_3$ | Thyroxine(µg/ml) $T_4$ |
|---|---|---|---|
| Normal control 10ml/kg 1% CMC | 2.70±0.31 | 1.41±0.21 | 53.42±5.22 |
| LT4 treated animals | 0.53±0.21 | 2.71±0.23 | 214.53±6.88 |
| STD control PTU 10mg/kg | 1.43±0.22 | 1.89±0.34 | 59.89±4.23 |
| MEAN 150mg/kg | 2.59±0.32 | 2.63±0.47 | 74.74±5.23 |
| MEAN 250mg/kg | 2.61±0.22 | 2.88±0.36 | 69.53±6.73 |

**Antioxidant Activity**

Increased lipoperoxidation (LPO) reactions and ROS production due to thyroid disease is widely established. The oxidative breakdown of cellular membranes is an autocatalytic mechanism called lipoperoxidation (LPO). The most significant of these free radicals is malondialdehyde (MDA), and its breakdown can lead to cell death and the creation of other toxic and reactive aldehyde metabolites. It is well established that reactive oxygen species

(ROS) can cause oxidative damage to biological macromolecules such as lipids, proteins, and DNA, and that oxidative stress can also influence adipocytes in the body, leading to a reduction in body fat mass and, consequently, in body weight. LPO ultimately results in MDA. Liver malondialdehyde (MDA) content has been shown to be an excellent predictor of LPO severity, and significant increases in MDA levels in the liver have been reported in hyperthyroid animals. Glutathione (GSH) is widely recognized as a protective antioxidant component in tissues; it prevents tissue damage by maintaining low ROS levels and specific cellular concentrations. Catalase is an enzyme that catalyzes the conversion of $H_2O_2$ to $H_2O$, and SOD is one of the antioxidant enzymes that contribute to enzymatic defense mechanisms. The rise of some antioxidant enzymes activities such as SOD and catalase may be symptomatic of the failure of balancing the generated oxidative stress. Marked decreases in tissue GSH concentrations were induced in hyperthyroidism, representing decreases of antioxidant defense systems. SOD and catalase activity increased to remove excess ROS, which some take as evidence of a failure to adequately compensate for the oxidative stress that had been artificially created. This study provides direct proof that MEAN have powerful antioxidant properties sufficient to suppress hyperthyroidisms by reducing LT4-induced oxidative stressors and the associated organ damages. We did not isolate any active components from the crud extract, but rather concentrated on its preventive benefits against hyperthyroidism in vivo. As a result, these kinds of proactive compound searches need to continue [32-34].

**Antihyperglycemic Activity**

The glucose oxidase method is commonly employed for the assessment of serum glucose levels. The process of glucose oxidation to gluconic acid is facilitated by the enzyme glucose oxidase. The hydrogen peroxide that has been produced is identified through the utilization of a chromogenic oxygen acceptor known as phenolaminophenazone, which is present alongside peroxidase [19, 20].

**Table 4: Serum glucose concentration in hyperthyroid**

| Sr. No. | Groups | Serum glucose concentration |
|---|---|---|
| 1. | Normal control 10 mg/kg 1%cmc | 41.20147 ± 0.478 |
| 2. | LT4 treated animals | 81.4017 ± 0.7894 |
| 3. | STD control PTU 10 mg/kg | 46.19333 ± 0.2547 |
| 4. | Mean 150 mg/kg | 52.87564 ± 0.3589 |
| 5. | Mean 250 mg/kg | 51.31 ± 0.4789 |

LT4 induced hyperthyroid rats has hyperglycemia because of stress. Since thyroid hormones are also gluconeogenic as well as glycolgenolytic in nature, the changes in serum glucose concentrations could be the result of methanolic extract of A. nilagirica induced alterations in the status of thyroid functions in animals.

**CONCLUSION**

In summary, it can be inferred that *Artemisia nilagirica* possesses the potential to serve as a valuable medicinal resource, displaying a diverse array of biological activities. Oral administration effectively reduced the development of hypothyroidism and liver damage generated by LT4. Furthermore, the researchers observed that the administration of the substance resulted in an enhancement of the liver's antioxidant defense systems. This was

evidenced by the dose-dependent inhibition of LT4-induced increases in lipid peroxidation and alterations in glutathione levels, superoxide dismutase activity, and catalase activity. These findings provide direct evidence that the substance has a beneficial ameliorating effect on hyperthyroidism and associated organ damage induced by LT4, likely through its antioxidant properties. The results of the study demonstrated that mean had similar effects on rat hyperthyroidism produced by LT4 when compared to PTU at a dosage of 10mg/kg. The aforementioned actions of mean have the potential to contribute to the amelioration of hyperthyroidism and its associated organ impairments. However, it is imperative that more investigations be conducted to identify and isolate the active compounds responsible for these effects.

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**Conflict of Interest**
None

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