Potential therapeutic properties of methanolic extracts of *Bacopa monnieri* for treatment ofhypothyroidism

Sangita Das*, Sagarika Dutta, Taslema Begum, Jogen Chandra Kalita

Department of Zoology, Gauhati University, Gopinath Bordoloi Nagar, Guwahati, 781014, Assam, India

*Corresponding author: Sangita Das.

Email: sangitadas28@gauhati.ac.in

Postal address: C/O Prof. Jogen Chandra Kalita,

Department of Zoology, Gauhati University, Gopinath Bordoloi Nagar, Jalukbari, Guwahati-781014, Assam, India.

HIGHLIGHTS

- Natural resources are huge reservoirs of therapeutically important compounds.
- *In silico* study was used to uncover thyroxine receptor inhibitors.
- Five molecules display thyroxine receptor inhibition potential from *Bacopa monnieri*.
- The identified molecules may reduce the detrimental effect of hypothyroidism.

ABSTRACT

The therapeutic application of natural products is yet to be investigated to their full potential. In the current study, identification of a few natural molecules from *Bacopa monnieri* methanolic extract was attempted, which may display notable interaction potential with the Thyroid hormone Receptor (TR) and Thyroid peroxidase (TPO). The triiodothyronine (T3) thyroid hormone binds with TR proteins after its secretion, thereby causing thyroid-mediated activities. An imbalanced release of T3 could cause detrimental activity within the human body. Therefore, an effective way to minimize this condition would be to synergize the TR and TPO proteins. A few probable candidates were obtained from *Bacopa monnieri* methanolic extract and performed molecular docking studies. Molecules such as 3, 5-bis (1,1-dimethyl ethyl), methyl 3-bromo-1-adamantane acetate, galactopyranoside, 1-octylthio-1-deoxy, 1, 2-benzenediol, thymol and carvacrol derivative showed notable TR and TPO binding efficiency. The binding efficiency was confirmed by the low binding energy and inhibition constant profile. They displayed amicable drug-like properties. The noted compounds showed no probability of hepatotoxicity, carcinogenicity, or mutagenicity. In addition to that,

all these compounds followed Lipinski parameters, which further supports their probability as test candidates for experimental study. The application of *in silico* study provides an important platform to expiate drug discovery research. Its cost-reduction efficiency compared to traditional trial and error methods increases the chance of developing molecules with a higher probability of success. In this case, the number of test candidates was narrowed down from 47 compounds to 5 probable candidates. All the selected compounds were effectively complying with drug discovery parameters. Later, it was found that the plant extract of *Bacopa monnieri* was enhancing the healthy follicles in rat models. Through this, it can be hypothesized that the selected compound in the extracts may show protective activity, and they could be further explored for their anti-hypothyroidism property.

Keywords: *Bacopa monnieri*, Anti-hypothyroidism, Docking, Plant extract, Rat model, Drug discovery

1. Introduction

Thyroid hormone-derived functions direct a wide array of functions within our body [1]. Activities such as overall development, physiological metabolism, cardiac functioning, and plenty more are significantly related to thyroid hormone functioning [1]. The gland secreting this hormone is present in the human neck region. It produces two major types of hormones such as triiodothyronine (T3) and thyroxine (T4) [2]. A higher concentration of T3 compared to T4 is responsible for the hormonal-derived roles. Here T3 is the active form, whereas T4 hormone is an inactive variant. Once secreted via the thyroid gland, T4 gets converted into T3, which leads to various functions [1]. Both hormones play a crucial role to maintain proper balance in the body. Once released these hormones are transported in the blood by binding to a protein called thyroxine-binding globulin (TBG) [1]. TBG protein is composed of 415 amino acid residues, which are attached with N-linked oligosaccharides (4 units) [1]. Thyroid hormone receptor (TR) helps in production of T3 from T4 in liver. TR also helps in regulation of TSH level in body by acting in the feedback mechanism of hypothalamic-pituitary-thyroid axis. There are instances that TR agonists are supposed to minimise the effect of metabolic diseases. As there are no publication on interaction between the compounds taken in the study with TR and thyroid peroxidase (TPO), the present study has been designed [3]. The production of T3 and T4 is largely affected by the activity of a protein known as Thyroid peroxidase (TPO). TPO or iodide peroxidase converts iodide to its atomic iodine, which interacts with tyrosine within a glycoprotein (thyroglobulin) that forms monoiodotyrosine (MIT) along with diiodotyrosine (DIT). Later, these iodinated proteins bind with thyroglobulin (Tg), that directs the formation of T3 and T4 [4].

Modulation in these hormones production, such as overproduction (hyperthyroidism) or less production (hypothyroidism), could be fatal for a person if not taken care of properly. Autoimmune disorders like

Hashimoto's thyroiditis and Graves' disease, which cause hypothyroidism and hyperthyroidism, respectively, may account for this modulation [5]. Hyperthyroidism could lead to an increase in metabolic rate thus in turn increase in heartbeat, blood pressure, insomnia, weight loss, muscle weakness, anxiety and if left untreated for a long could lead to bone loss. Its treatment involves using drugs such as methimazole or propylthiouracil to reduce hormone production or radioactive iodine to destroy thyroid cells [6]. While hypothyroidism could lead to a reduction in metabolic rate thus in turn leading to weight gain, muscle weakness, slow heart rate, constipation, fatigue, cholesterol, heart disease and an enlarged thyroid gland (goiter). Treatment for hypothyroidism involves administering synthetic thyroid hormone such as levothyroxine [7]. T4 gets converted into its active form, T3 in tissue such as liver and kidney, wherein it enhances the basal metabolic rate, oxygen consumption, and heat generation within the body. They promote the breakdown of glycogen and glucose, enhance glucose uptake by cells, and stimulate the metabolism of fats to generate energy. Thyroid hormones also promote the synthesis of new proteins, which are essential for growth and tissue repair.

The current study is an attempt to uncover natural molecules that may possess the ability to block the TR receptor and TPO protein. The objective was to examine the impact of a methanolic extract derived from *Bacopa monnieri* on addressing hypothyroidism in Albino rat models. This plant has previous reports to show anti-hypothyroidism [8]. The study aimed to establish a relationship between the activity of specific compounds, their interaction with TR receptor, and their potential to synergize the combined activity of T4 and T3 hormones. Through this strategy we target to hypothesize a possible application of natural molecules to counter hypothyroidism. Molecular docking analysis was performed with a series of compounds against T3-Bound Thyroid Hormone Receptor protein (PDB ID: 3GWS) and TPO protein. A set of five compounds were the best-performing molecules that displayed notable binding efficiency, drug-like property and negligible toxicity. Therefore, the application of *in silico* studies supported by *in vivo* experiments may lead us to uncover newer molecules that may display notable therapeutic activity to alleviate hypothyroidism.

2. Materials and Methods

Preparation of Plant Extract

Bacopa monnieri (Plantaginaceae) plant which is locally known as Brahmi in native language was used for the study [9]. Fresh leaves of the plant were collected, and a representative sample was authenticated in the Department of Botany, Gauhati University, Assam. The sample was marked with accession number (#19765), and a sample copy was preserved for future reference. Prior to extraction, leaves were thoroughly washed followed by shade drying and storage. Dried leaves were coarsely ground and treated with methanol for extraction using a cold maceration technique (72 hours) [10]. *Bacopa monnieri* methanolic extract (BMME) was filtered using Whatman filter paper, followed by concentration using a rotary evaporator at

reduced pressure and temperature. Dried samples were stored in low temperatures and reduced moisture for future uses.

Gas Chromatography-Mass Spectroscopy (GC-MS) Analysis

The methanolic extract of *Bacopa monnieri* (L.) Wettst. was subjected to GC-MS analysis to identify the constituents present. 1 mg of the methanolic extract was dissolved in 2.0 ml of DMSO (analytical grade). Next, the sample was centrifuged at 10,000 rpm for 5.0 minutes. The resulting clear solution was then transferred to the autosampler. The injection volume was adjusted to 3.0 µl. Two mobile phases were employed, namely A (0.1% formic acid in water) and B (0.1% formic acid in acetonitrile), at a flow rate of 500 µl min^{-1}.

A well-defined protocol was implemented to conduct GC-MS analysis. During the analysis, 99.99% helium was used as the carrier gas, flowing at a rate of 1 mL/min. The injection volume was set to 1 µL in splitless mode. The injector temperature was maintained at 280°C, while the ion-source temperature was set to 180. The samples' mass spectra were acquired using Electron Impact positive (EI+) mode with an energy of 70 electron volts (eV). A solvent delay of 8 minutes was employed before conducting the mass spectrometry (MS) scan. The analysis was conducted within a mass range of 50 to 600 atomic mass units (amu). The corresponding peak's mass spectrum was subjected to a library search using the NIST-2014 database software to identify the peaks in the GC chromatogram. The unknown mass spectra were compared to the recognized compounds in the NIST library to determine the empirical formula, molecular weight, name, as well as other relevant information of the compounds.

Synthesis of Ligands and Receptors

Collectively, 47 compounds from GCMS data of *Bacopa monnieri* were selected for our investigation (**Supplementary Table 1**). The PubChem database was used to obtain the Canonical SMILE format of the 47 compounds, as shown in **Supplementary Table 2**. Next, the structure builder option of Chimera was employed to construct their structures. Before the experiments, the freely available UCSF Chimera version 1.14 software [11] was utilized for geometry optimization. The resulting 3-D structures were saved in protein databank coordinate files (.pdb) format. The crystal structure of the Thyroid Hormone Receptor bound to T3 (PDB ID: 3GWS) was acquired from the protein data bank (PDB) repository, accessible at www.rcsb.org. The wizard (Version 2021-2, Schrodinger) for protein preparation [13] was utilized to process and refine the structure. The cofactors, ions, and water molecules were eliminated, and hydrogens were added to the heavy atoms. Selenomethionines were transformed into methionines. Epik was employed to generate Het states at pH 7.0 ± 2.0, and protein minimization was conducted using the OPLS_2005 force field [11]. The three-dimensional (3-D) structure of Human Thyroid Peroxidase (TPO) was created through homology modeling using the SWISS- MODEL web server (https://swissmodel.expasy.org/). The protein

sequence was obtained from the UniProt database (Entry ID: P07202) in FASTA format. The Swiss Model structure assessment tool (https://swissmodel.expasy.org/assess) was employed to evaluate the quality of the generated model.

Drug-like properties and toxicity assessment

The screening of compounds was conducted using SwissADME (http://www.swissadme.ch/), which calculated drug-likeness properties based on Lipinski [14, 15], Ghose [16], and Veber [17] criteria, as outlined in a previous study [12]. The toxicity (Hepatotoxicity, Carcinogenicity, and Mutagenicity) of the compounds were investigated utilizing the ProTox-II webserver (http://tox.charite.de/protox_II) [13].

Molecular Docking

The molecular docking study utilized the crystal structure of the Thyroid Hormone Receptor bound to T3 (PDB ID: 3GWS) and the Human Thyroid Peroxidase (TPO). Autodock 4.2 was employed for conducting all the protein-ligand docking studies.[14]. The molecular dockings were carried out according to the previously reported studies [15-17]. The amino acid residues in the catalytic site of the T3-Bound Thyroid Hormone Receptor protein were taken from the previously published results [18]. The receptor protein was maintained in a rigid conformation, whereas the ligand compounds were allowed to be flexible. The protein and ligands were supplemented with polar hydrogen and Gasteiger charges. To prepare PDBQT files of the ligands and proteins, AutoDock Tools (v.1.5.6) from the MGL software package was utilized. The protein-ligand docking complexes were analyzed using the Lamarckian genetic algorithm (LGA) method.

The dimensions of the grid box were configured at 80 × 64 × 72 and 126 × 126 x 126 xyz points, respectively, for T3-Bound Thyroid Hormone Receptor protein and a model of TPO was constructed with 0.375 Å grid spacing. The parameters were employed for performing grid map calculations for both enzymes. The grid center coordinates for the T3-Bound Thyroid Hormone Receptor protein were set at 3.634 Å (x), 19.698 Å (y), and 31.3 Å (z). As for the TPO build model, the grid center coordinates were established at 20.951 Å (x), 8.082 Å (y), and 13.832 Å (z). These values were utilized in the grid map calculations for both enzymes. The program executed 100 Genetic algorithm runs, with default settings for all other parameters. The results of the molecular docking study provided binding energy information, including the docking score and binding interactions of each ligand with the protein target under investigation. The interactions between the docking complexes were examined, and the resulting 3D and 2D interaction plots were analyzed using UCSF Chimera version 1.14 software. [11] and Accelrys Discovery Studio Visualizer (Dassault Systèmes BIOVIA 2017), respectively [19].

2.6 *In vivo* anti-hypothyroidism in rat models

The Female Wistar albino rats were randomly and equally distributed into six groups. Apart from animals in a normal control group, the rest were treated with methimazole (60 mg/ Kg) to induce hypothyroidism. The test groups were treated with either BMME (200 mg/ Kg) or 0.02 mg L-thyroxine for 14 days and 21 days. Later the animals were sacrificed, and sections of tissue (thyroid glands) samples were prepared [20]. Isolated glands were fixed with neutral buffered formalin, followed by graded dehydration using an ascending concentration of alcohol (30-100 %). Later, the tissue samples were treated with xylene, and they were embedded in paraffin blocks. Samples were sectioned using a microtome. After that, samples were deparaffinized using xylene, followed by staining using Eosin Hematoxylin solution. The stained samples were mounted in DPX and examined using a microscope [20]. All the experimental steps were conducted as per the procedure of the Committee for Control and Supervision of Experiment on Animals (CPCSEA) and the Institutional Animal Ethical Committee (IAEC).

3. Results

Gas chromatography-mass spectrometry (GC-MS) analysis

GC-MS chromatogram investigation of the methanolic extract of *Bacopa monnieri* (L.) Wettst. depicted peaks that indicate the presence of phytochemicals (**Figure 1**). Upon examining the mass spectral profiles of the components using the MassBank library, the identification of phytocompounds was accomplished. The molecular structures of various compounds from *Bacopa monnieri* (L.) Wettst. leaves are shown in Fig. 3. But the structure of Acacetin-6,8-di-C-hexoside was not available in any database. It has been established through prior research using LC-ESI-QTOF-MS that isomeric jujubogenin and pseudojujubogenin aglycones are present in the ethanolic preparation of *Bacopa monnieri* [12].

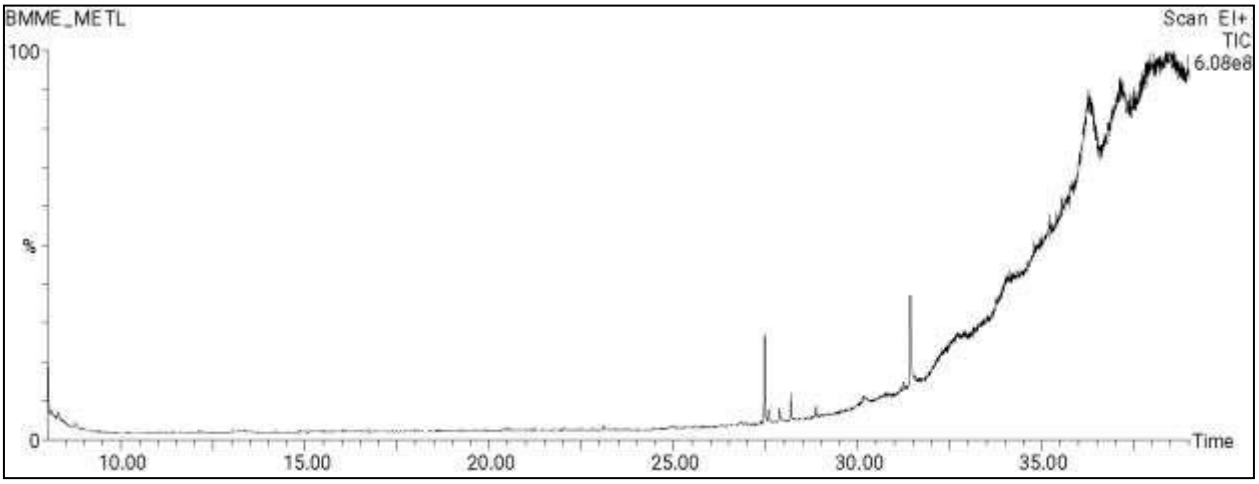


Figure 1: GC–MS chromatogram of methanolic extract of *Bacopa monnieri (L.)*

Structure Assessment of Thyroid peroxidase Protein (TPO)

The structure of the Human Thyroid was created using Myeloperoxidase as a template [27]. This enzyme is responsible for thyroid-mediated activity such as metabolism and overall development [21]. This protein is categorized as a nuclear hormone receptor [22]. The functional structure of this protein was analyzed and reported by Nascimento et al. which showed detailed rearrangement and active domain site within the protein [22]. The Ramachandran plot obtained in this study showed the dihedral angles of amino acids composing TPO protein. Template Myeloperoxidase (5uzu. 1. B) was used to build the TPO protein (sequence identity 47.99 %, Ramachandran favored (95.90%) and Ramachandran Outlier (0.51%) (**Figure 2**).

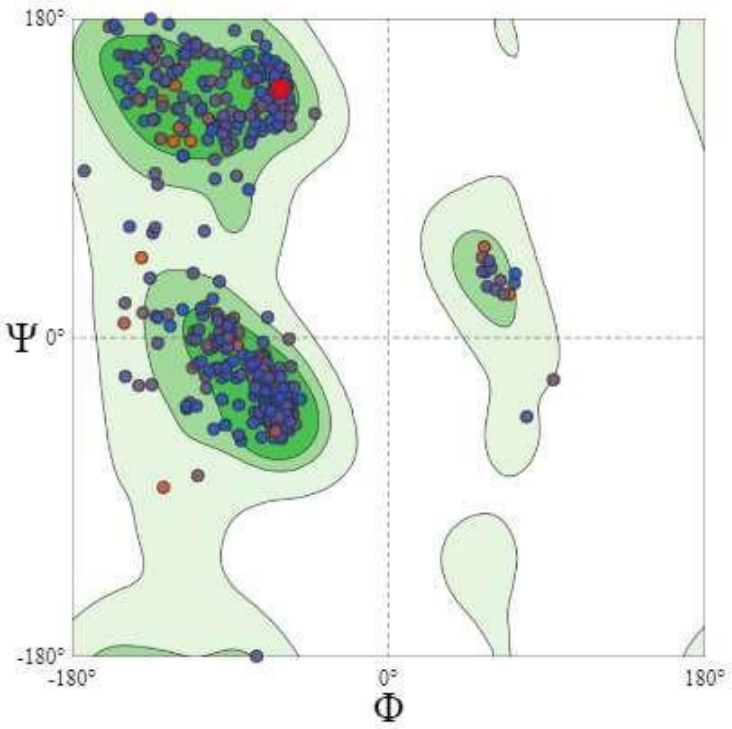


Figure 2: Ramachandran plot showing the dihedral angles of amino acids composing TPO protein.

In silico Physicochemical, ADME Parameters, and Toxicity analysis

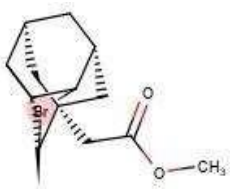
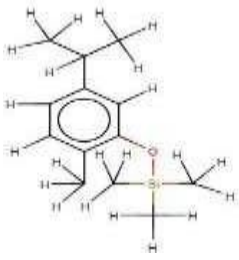
Swiss ADME was employed to assess the Absorption, Distribution, Metabolism, and Excretion (ADME) properties of the corresponding compounds. Lipinski's rule of five and the Ghosh and Veber filters were applied during the evaluation process. For each ligand, drug-likeness properties such as LogP, MW (molecular weight), the number of hydrogen-bond acceptors (HBA), the number of hydrogen-bond donors (HBD), MLOGP, WLOGP, MR (molar refractivity), and the total number of atoms were predicted. The results of the respective compounds are presented in **Supplementary Table 1**. Among the 47 compounds assessed, 5 compounds successfully met all the established criteria without any violations. All these 5 compounds also demonstrated high gastrointestinal (GI) absorption. The webservers ProTox-II was used to

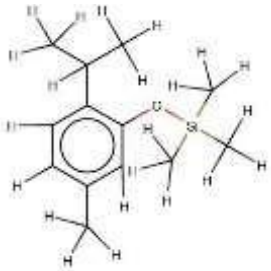
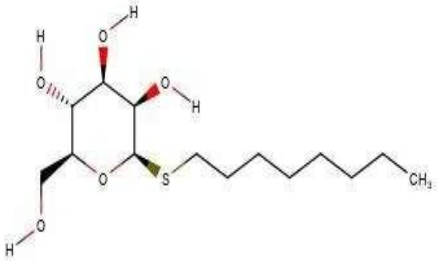
predict toxicity parameters. The toxicity outcome revealed that 5 compounds chosen out of 47 showed no hepatotoxicity, carcinogenicity, and mutagenicity (**Supplementary Table 1**).

Molecular docking studies

Molecular docking analyses were conducted to unravel the binding affinity of the compounds with the T3-Bound Thyroid Hormone Receptor (PDB ID: 3GWS) and Human Thyroid Peroxidase (TPO) protein (**Supplementary Table 1**). The docking results showed that methyl 3-bromo-1-adamantaneacetate, carvacrol, thymol, galactopyranoside-1-octylthio-1-deoxy and 1,2-benzenediol, 3,5-bis (1,1-dimethylethyl) be the prominent inhibitory compounds with the high binding affinity with drug-likeness properties and no toxicity (**Supplementary Table 1**). The images of docked complexes are shown in **Figures 3 and 4**. Molecular docking studies revealed that these compounds bind significantly with T3-Bound Thyroid Hormone Receptor protein with the lowest binding energies varying from -6.8 to -7.44 kcal/mol and between -5.8 to 7.75 kcal/mol with human Thyroid peroxidase (TPO) protein, respectively.

Table 1: 2-D structures of compounds exhibiting the protein-ligand complex formation ensuing the most favorable binding energies.

S. no	Chemical structure	Compound name
1		Methyl-3-bromo-1-adamantaneacetate
2		Carvacrol, TMS derivative

3		Thymol, TMS derivative
4		Galactopyranoside, 1-octylthio-1-deoxy
5		1,2-Benzenediol, 3,5-Bis(1,1-dimethylethyl)

The 2-D images of molecules in **Table 1** depict mostly non-bonded interactions, such as van der Waal's, a few hydrogen bonds, π bond, and interaction between alkyl groups and the amino acid residues. This study reveals the detailed interaction of the selected compound and its bonding interaction with the specific amino acids within the receptor protein (PDB ID: 3GWS) (**Figure 3, Supplementary Table 3**). Methyl-3-bromo-1-adamantaneacetate had shown the interaction with asparagine 130. Whereas carvacrol and thymol derivatives did not show specific interactions. Similarly, galactopyranoside, 1-octylthio-1-deoxy showed interaction with arginine 81, asparagine 130, arginine 115 and threonine 128. The compounds 1,2-benzenediol, 3,5-Bis (1,1-dimethylethyl) showed the interaction pattern with phenylalanine 71. The binding interaction of the compounds is confirmed by their binding energy (Kcal/ mol) and inhibition constant profile (Kcal/ mol) (**Supplementary Table 1**). These compounds showed lower values of both parameters.

The lowest values obtained by this compound are directly creatable with their higher probability of interaction.

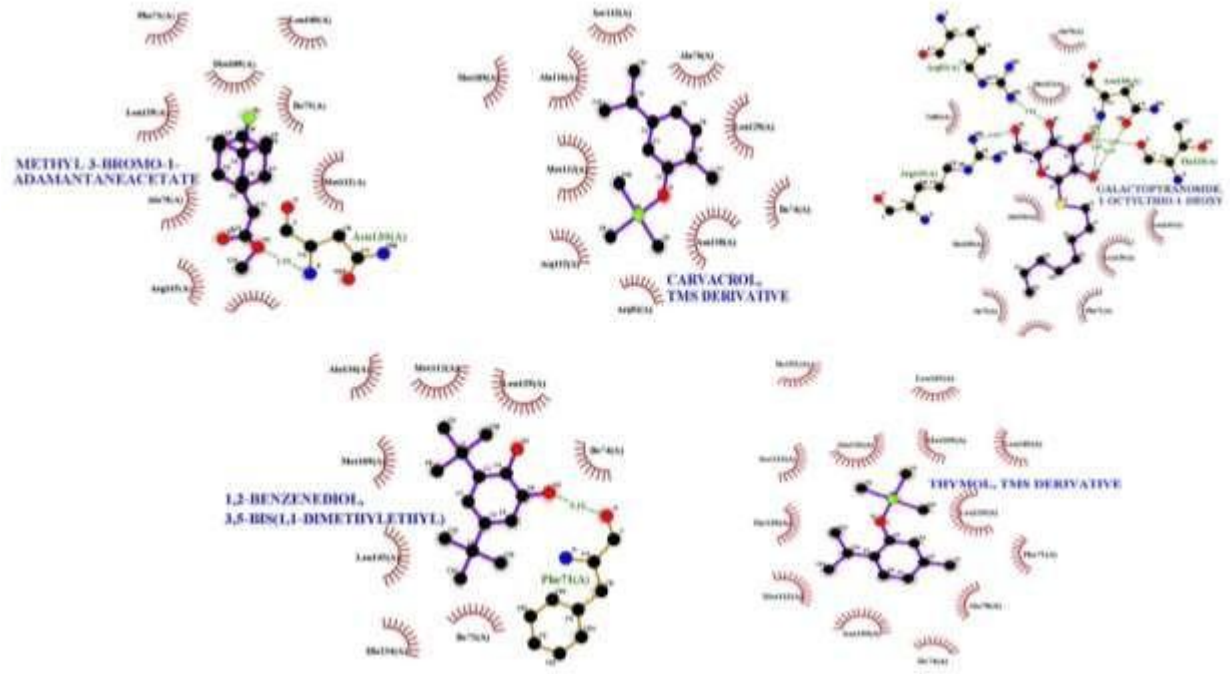


Figure 3: Molecular docking interaction of the compounds with T3-Bound Thyroid Hormone Receptor (PDB ID: 3GWS) corresponded by the 2D illustration of the ligand interactions with amino acid residue.

Similarly, the detailed analysis of human thyroid peroxidase interaction with the selected compounds was studied (**Figure 4, Supplementary Table 4**). The methyl-3-bromo-1-adamantane acetate interacts with arginine 582, whereas carvacrol and thymol showed interaction with histidine 494. Galactopyranoside, 1-octylthio-1-deoxy displayed interaction with three amino acids (phenylalanine 490, glycine 493 and histidine amino acid) with 1,2-benzenediol, 3,5-Bis (1,1-dimethylethyl) had shown interaction potential with Asparagine 170. The binding of the compounds to the receptor position is decided based on their physiochemical properties. The *in-silico* studies indicate the probability of drug-receptor interaction, which may be examined in the actual model before an absolute claim. Our study suggests that the selected compound has a specific interaction profile within the receptor site. All the compounds showed their interaction with different amino acids with a few exceptions.

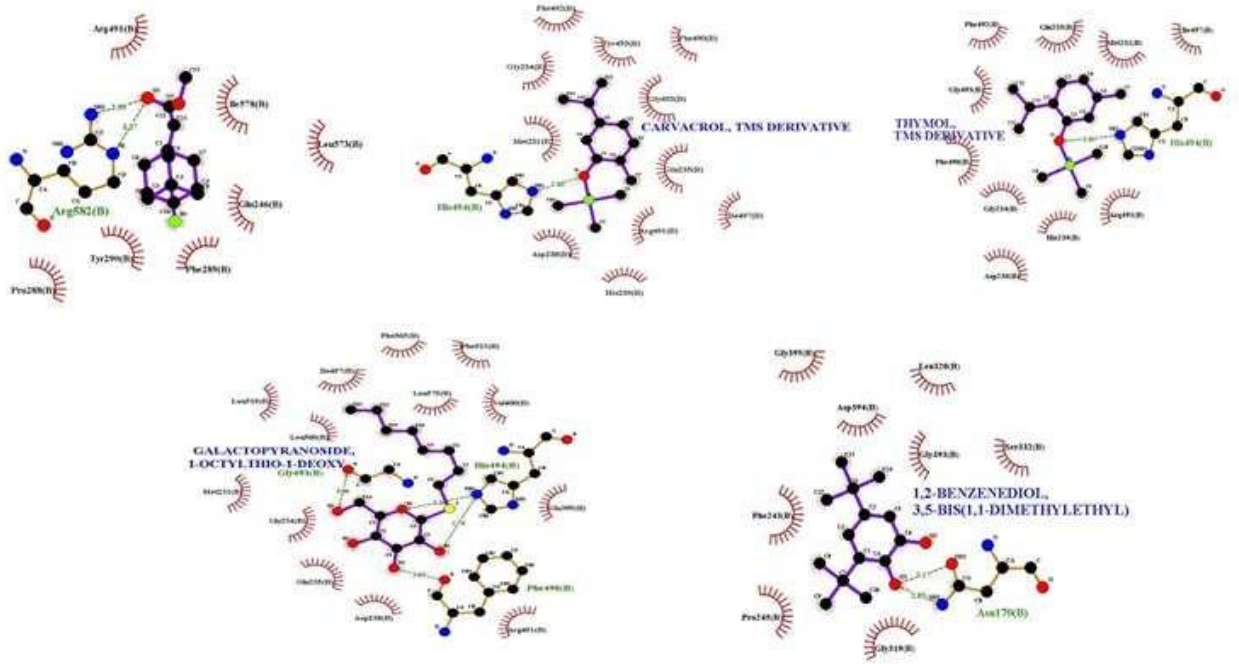


Figure 4: Molecular docking relations of the compounds with human Thyroid peroxidase (TPO) corresponded by the 2-D depiction of ligand interactions with amino acid residues.

In vivo activity

Female Wistar albino rats as an animal model were used to detect the anti-hypothyroidism potency of the *Bacopa monnieri* plant extract. Hypothyroidism was induced by administering oral doses of methimazole prior to the treatment of the test extract. After the duration of treatment, animals were sacrificed, and tissue sections were prepared for detailed examination. Light microscopic study in the control group showed normal histoarchitecture of the follicles and thyrocytes (**Figure 5**). The follicles in this group showed spherical architecture containing eosinophilic colloids. Animals in the methimazole-treated group did not show normal histoarchitecture. Follicles displayed irregularly shaped and large vacuoles appeared within these groups of animals. BMME treated group (200mg/kg body weight) for 14 days showed very few normal follicles, which indicates a lowered degree of healing potential. It showed a comparable profile to the hypothyroidism-induced control (methimazole) group. A few large vacuoles were observed at a lesser frequency when compared to methimazole-treated animals.

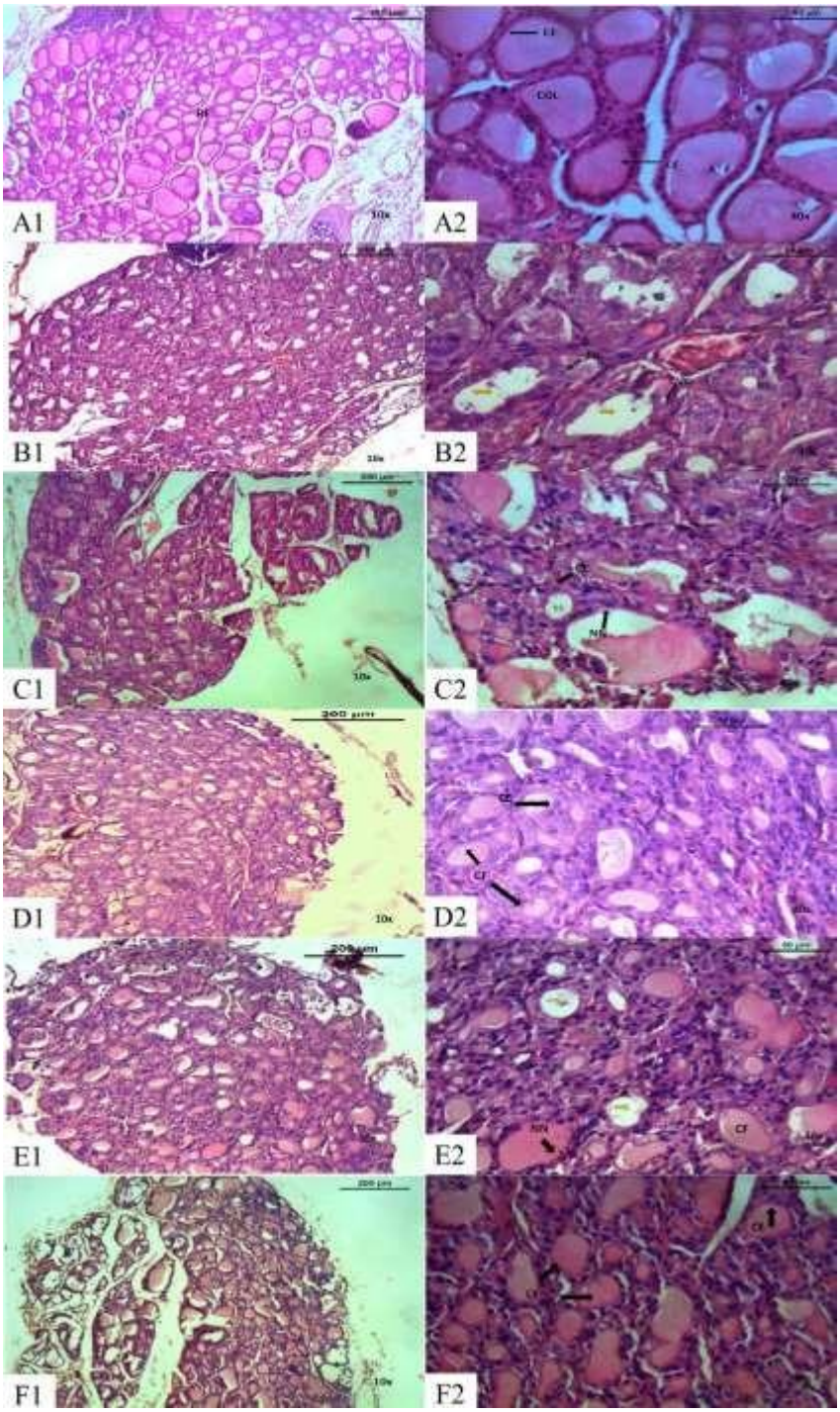


Figure 5: Histology analysis showing tissue section of the thyroid gland after standard drug and test extract in hypothyroidism-induced rats. A1 (10X) and A2 (40X) are normal control groups, whereas B1 (10X) and B2 (40X) are methimazole control groups. The BMME group (200 mg/ Kg) is shown by C1 (10X) and C2 (40X), whereas D1 (10X) and D2 (40X) are animals treated with L-thyroxine for 14 days. The 21 days

treated BMME are E1 (10X) and E2 (40X), whereas similar duration of L-thyroxine is shown as F1 (10X) and F2 (40X)

However, the thyroid follicles were seen to recover during the 21 days treatment regimen. Most of the follicles were observed to be normal size with a colloid-filled lumen. There were no unusually large vacuoles along with the follicles that seemed to attain regular geometry as observed in the case of the untreated control group. The standard treated control group (L-thyroxine) displayed notable recovery activity even in 14 days of treatment duration, which led to better healing when treatment was extended to 21 days. Overall, the recovery potential of BMME extracts seems to be comparable with that of 14-day treated L-thyroxine group. Whereas 21 days L-thyroxine treated group displayed maximum healing potential. The activity profile of BMME extract is still noteworthy as the plant extract showed recovering potential, where the quantity of active molecules is significantly lower.

4. Discussion

Natural flora and fauna contain numerous molecules with notable therapeutic activity [23]. There have been reported molecules from natural resources used in the current therapeutic regimen [23]. Among the wide collection of herbs, a plant named *Bacopa monnieri* is known to possess beneficial effects in neuropharmacological applications. *B. monierri* is reported to display notable neuropharmacological benefits related to boosting overall memory in patients possessing Alzheimer's disorder or as an antiparkinsonian agent. It is also known to display protective activity against schizophrenia, convulsion or anti-stroke potentials [24]. The activity profile is often related to the presence of triterpenoid saponins commonly known as bacosides. These molecules have been reported to increase neuronal transmission, repair damaged neurons, repair synapses which enhance the overall mental activity profile [24]. This herb is especially known to display curative effects in case of thyroid disorders [8]. Thyroid hormone directs important physiological functions. However, improper release of this hormone has profound effects on the overall metabolism and growth of an individual [5-7, 22]. A thyroid gland that is underactive, or one that does not produce enough thyroid hormones to meet the body's requirements, is the hallmark of the medical illness known as hypothyroidism. Hypothyroidism can arise because of primary gland failure or insufficient thyroid gland stimulation by the pituitary or hypothalamus [25].

In the present study, the therapeutic potential of methanolic extracts derived from *Bacopa monnieri* was assessed to treat hypothyroidism. It was observed that the BMME extract exhibited a protective effect on thyroid follicles when administered at a dose of 200 mg/kg body weight administered for 21 days in the hypothyroidism-induced rat models. The observed activity profile was comparable to the 14-day L-thyroxine treatment group. However, the 21-day L-thyroxine-treated group exhibited the most favorable activity profile. In a study conducted by Sharma et al. on male mice, it was observed that Brahmi (*Bacopa*

monnieri) exhibits thyroid-stimulating activity [26]. In a similar study, rats with hypothyroidism were administered 200 mg per kg of Brahmi and the plasma levels of thyroid hormones (T3, T4, and TSH), lipid profile, and liver antioxidants were evaluated. It was found that Brahmi effectively improved hypothyroidism by reversing various biochemical changes and restoring the histology of the thyroid gland in rats [27]. These findings suggest that Brahmi holds potential as a therapeutic choice for managing clinical conditions linked to hypothyroidism.

The docking results of *Bacopa monnieri* compounds in this study revealed that methyl 3-bromo-1-adamantaneacetate, carvacrol, thymol, galactopyranoside-1-octylthio-1-deoxy, and 1,2-benzenediol, 3,5-bis (1,1-dimethylethyl) exhibited notable inhibitory properties with high binding affinity. These compounds demonstrated drug-like properties and showed no signs of toxicity. In addition, these compounds exhibited significant binding to the T3-Bound Thyroid Hormone Receptor protein (TR) and to the human Thyroid Peroxidase (TPO) protein. The compound methyl-3-bromo-1-adamantane acetate was found to interact with arginine 582, while carvacrol and thymol demonsβated interaction with histidine 494. Galactopyranoside, 1-octylthio-1-deoxy displayed interactions with three amino acids, namely phenylalanine 490, glycine 493, and histidine amino acid. On the other hand, 1,2-benzenediol, 3,5-Bis (1,1-dimethylethyl) exhibited interaction potential with asparagine 170. The binding positions of these compounds to the receptor site were determined based on their physiochemical properties.

It is important to note that these in-silico studies provide insights into the likelihood of drug-receptor interactions, serving as an initial indication. The study suggests that the selected compounds exhibit a specific interaction profile within the receptor site, each showing interactions with different amino acids, with a few exceptions. These findings highlight the potential of these compounds to modulate the activity of TPO and contribute to a better understanding of their mechanism of action in the context of thyroid-related conditions. By conducting an in-silico analysis of the intrinsic secondary metabolites found in *Bacopa monnieri* and their interactions with relevant proteins, potential curative effect for hypothyroidism was identified. Upon experimental evaluation, significant restorative potential was observed in the methanolic extract of *Bacopa monnieri* compared to treatment groups receiving standard molecules such as L-thyroxine. These findings underscore the importance of systematic research methodology in predicting and uncovering the therapeutic profiles of unexplored natural resources.

The present study also suggests that the BMME treatment had limited restorative effects on the histopathological changes caused by hypothyroidism. The presence of vacuoles in the BMME-treated group generates scope for further investigation to determine the optimal dosage and duration of treatment for achieving more significant therapeutic outcomes. It is worth noting that the activity profile of the BMME extract is noteworthy considering that the quantity of active molecules in the extract is significantly low.

Despite this, the plant extract exhibited considerable recovery potential, indicating its potential therapeutic value in hypothyroidism treatment. Further research is warranted to explore the specific active components within the BMME extract and optimize the dosage and treatment duration for enhanced therapeutic effects.

Authorship Contribution

SD (First author) formulated the concept for the study, executed the experiments, analyzed the data, and prepared the draft of the manuscript. **SD** (Second author) & **TB** conducted the molecular docking studies and **JCK** Supervised the entire work. All the authors assisted in improving the manuscript.

Declaration of Competing Interest

There is no conflict of interest among the authors.

Data Availability

Data will be made available on reasonable request to the author.

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