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2d-Qsar and Molecular Docking based Screening and Anti-Arthritic Activity of Ethanol and Aqueous Extract of *Gmelina Arborea*

Madan Lal Kaushik[1], Poonam Dogra[1*], Mahendra Singh Ashawat[2]

[1]Adarsh Vijendra Institute of Pharmaceutical Sciences, Shobhit University, Gangoh, Saharanpur- 247341, Uttar Pradesh, India

[2] Laureate Institute of Pharmacy SH 22, Kathog, District Kangra, Himachal Pradesh 177101

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KEYWORDS

Gmelina arborea, Rheumatoid arthritis. QSAR. Molecular docking, Inflammation. Herbal therapeutics.

ABSTRACT:

Gmelina arborea has been employed for treating rheumatoid arthritis in various traditional systems of medicine. The current study's objective was to use in-silico and in-vivo research to give a scientific basis for G. arborea's ability to treat arthritis. We created a strong 2D-QSAR model to forecast the anti-inflammatory properties of herbal compounds, and the results showed that the compounds found in G. arborea, specifically luteolin, apigenin, stigmasterol, and campesterol, were the most promising ones with strong anti-inflammatory properties. The findings were confirmed by the Molecular Docking investigation, which showed that these compounds had a strong binding relationship with the Janus kinase 3 protein. The Potential of ethanolic extract to reduce inflammation and arthritis and aqueous leaf extract of G. arborea was performed on female Wistar rats. Our results revealed that plant extract is having significant anti-arthritic potential as indicated by the reduction in inflammation and joint stiffness, along with improvement in gait, mobility, and visual weight bearing test along with significant improvement in the hematological profile. It was discovered that the aqueous extract's outcomes were more encouraging than the ethanolic extract's. Our research revealed that G. arborea has high potential as an arthritis preventive, which may be related to the plant's luteolin, apigenin, stigmasterol, and campesterol contents.

INTRODUCTION

Chronic ubiquitous autoimmune disease known as rheumatoid arthritis (RA) is typified by inflammation of the peripheral joints, cartilage destruction in the joint deformities, and stiffness in the synovial joints. (Miao *et al.*, 2021; Asif *et al.*, 2023; Srinivas *et al.*, 1994). The pathology of RA can be understood through the infiltration of leukocytes into the synovial membrane, which leads to persistent inflammation, abnormal granulation tissue layer on the joints, and comprehensive damage to the joint tissues and bone (Kshirsagar *et al.*, 2014; Borchers*et al.*, 2004). Although the exact etiology of RA is not fully understood, It is widely known that inflammatory cytokines, such as interleukins (IL-1β & IL-6) and tumour necrosis factor (TNF-α), have a role in the onset and progression of RA. (Yeom*et al.*, 2006).

Currently available anti-arthritic drugs are capable of providing only symptomatic relief from the disorder and RA continues to progress slowly and steadily despite regular therapeutic. Moreover, therapeutic potential (Narendhirakannan*et al.*, 2007). Plant-based therapeutic strategies for targeting RA has gained interest in recent time, considering the safety and vast potential of herbs as a source of bioactive molecules (Thomford *et al.*, 2018). *Gmelina arborea*, commonly known as "Gambhari," holds a significant place in traditional medicine for its potential role in alleviating RA symptoms. The plant's growl leaves, and root extracts have been utilized in various indigenous systems of medicine, such as Ayurvedic medicine and traditional Thai medicine, for their antiinflammatory and analgesic properties. Because they display anti-inflammatory effects via altering cytokine pathways and suppressing inflammation mediators, bioactive substances such as flavonoids, steroids, and terpenoids are thought to be responsible for these qualities. (Pongprayoon *et al*., 2003; Mahesh *et al*., 2010). Some studies have explored the plant's bioactive

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components and their potential to inhibit pro-inflammatory pathways, which could contribute to its traditional use for RA relief (Pandey *et al*., 2018). However, scientific research on *G. arborea*'s effectiveness in treating RA is limited, and its mechanisms of action are not fully understood. While traditional knowledge suggests *G. arborea* may offer benefits for RA, further rigorous research is required to provide an experimental validation to its traditional use.

In drug discovery, computational methods like Molecular docking and 2D Quantitative StructureActivity Relationship (QSAR) play pivotal roles in predicting and optimizing the interactions between potential drug compounds and target biological molecules. These computational techniques significantly accelerate the drug discovery process by narrowing down the pool of potential compounds for synthesis and testing. They offer cost-effective alternatives to highthroughput screening and can guide chemical modification for enhanced efficacy and reduced toxicity (Patel *et al*., 2008*)*. Incorporating 2D QSAR and molecular docking into drug discovery workflows facilitates the rational design of safer and more effective therapeutic agents, streamlining the journey from initial hit identification to optimized lead compounds. These computational techniques streamline the drug discovery process by reducing the need for extensive experimental screening, thus saving time and resources (Thakur *et al*, 2023; Mehta *et al.*, 2023).

In this investigation, we aimed to provide a scientific and experimental justification for the traditional use of *G. arborea* against RA by employing *in-silico* and *in-vitro* approaches. We employed a 2D-QSAR and molecular docking technique model to forecast the anti-inflammatory probable of the bioactive compounds of *G. arborea*, which was followed by an *in-vivo* investigation of the plant extract against RA in Wistar rats.

MATERIALS AND METHODS

Computer Hardware and Software

For the in-silico tests of 2D-QSAR and molecular docking, a Lenovo ThinkPad machine with an 11th generation Intel Core i5 @ 2.40 GHz processor, 8 GB RAM, and a 64-bit OS system was utilised. The Marvin Sketch (Chemaxon) was used for molecular modeling, drawing, and optimization of the structures of bioactive molecules. PAADEL-descriptor software was used for molecular descriptor calculations for QSAR model generation and the 2D-QSAR model was generated and validated with the help of QSARINS of the University of Insurbia. Molecular docking studies were performed by employing Auto Dock tools of The Scripps Research Institute.

QSAR analysis

Collection of Dataset and Optimization

The 2D-QSAR model was developed by utilizing twenty-six synthesized pyrimidino benzothiazole amine derivatives reported already in the literature (Doma *et al*., 2014). Following four hours of test compound administration, the percentage of rat paw edoema that was inhibited by each compound was reported as the biological activity (inhibition of rat paw edoema). The chemical structures of pyrimidino benzothiazole amine derivatives along with their biological activity are given in Supplementary Table I. The structures were prepared in mol format and their energy minimization was done by using Marvin Sketch.

Molecular descriptor calculations

Molecular descriptor calculation is an important step in the development of a robust QSAR model. In the current study, PADEL-descriptor software was used for calculating molecular descriptors of 26 derivatives (Yap *et al.*, 2011). This software computes a total of approximately 1875 descriptors which are wide enough for the development of a reliable, efficient, and robust 2DQSAR model.

Data pretreatment, dataset division, and QSAR model generation

It is essential to eliminate any constant and inter-correlated descriptors in order to prevent them from impeding the creation of an effective QSAR model, since this will ensure the model is resilient and dependable. Variance threshold values of 80% and 75% were used to exclude constant and inter-correlated descriptors, respectively. When developing a QSAR model, the genetic algorithm (GA) approach is a heuristic method that simulates the process of natural selection. In the current work, the QSAR equation is developed using this

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GA approach. The 70% training dataset and 30% test dataset were divided using a random division technique. The University of Insurbia's QSARINS software was used for each of these procedures. (Gramatica *et al*., 2013).

Internal Validation

To validate the trustworthiness of the QSAR model internally, a cross-validation method was used and Q^2_{cv} is calculated by using the following equation:

$$Q2cv = 1- [\Sigma(Y-Ypred)2/ \Sigma(Y-Ymean)2]$$

where Y_{mean} is the mean of the observed values in the training set, Y_{pred} is the value that the model predicts, and Y is the observed value (PIC50).

The produced QSAR model's internal predictability was previously evaluated using R^2 value, which gained popularity. Whenever a large number of descriptors are utilised, this parameter's worst flaw is that it always produces false positives. This was addressed by using the following equation to get the new parameter R^2_{adj}:

$$R^2_{adj} = R^2\text{-}p(n-1)/n\text{-}p+1$$

where n is the number of molecules utilised in the training set for model construction and 'p' is the number of descriptors employed. The difference between R^2 and R^2_{adj} should be less than 0.3 for a model to be considered acceptable.

External Validation

Supplementary Table II demonstrates the statistical properties that Golbraikh and Tropsha suggested for the resilient QSAR model with strong prediction performance (Golbraikhet al., 2002). where R'20 represents the relationship between the expected and observed values of the test set and R20 is the coefficient of squared correlation between the observed and predicted values.

Applicability Domain

The chemical structure space in which a QSAR model makes the most accurate predictions is known as the Application Domain (AD). According to the Organisation for Economic Cooperation and Development's third principle, defining the AD of a QSAR model is a requirement (OECD). Any created QSAR model's structural and response outliers may be found using this method, which is highly helpful. For the purpose of this study, we defined the AD of the present QSAR model using the Wiliams plot that Gramatica suggested. (Gramatica*et al*., 2007). The following formula is used to create a new parameter leverage value, h_i:

$$hi = xit(XtX)xi$$

The descriptor vector of the considered data point is represented by x_i, the descriptor matrix by X_{is}, and the transpose of the descriptor matrix by X_t. Using the following formula, the threshold leverage h^* was determined:

$$h^* = 3(p+1)/n$$

Where '*p*' depicts the number of variables, n depicts the number of compounds in the training set.

For a chemical to be within the AD, it's h_i should be less than h^*. The molecule having a small standardized residual but $h_i > h^*$ may not be considered an outlier but instead considered an influencer. The cut-off value for standardized residuals in defining AD is considered a value of ±3.

Screening of herbal molecules reported in *G. Arborea*

Screening of the 12 compounds reported from the plant *G. arborea* was done for the anti-arthritic activity. The probable percentage inhibition of the edema of these 12 compounds was calculated using the QSAR model generated. This computation is performed using the DTC-QSAR v1.0.6 software from the DTC lab. (Roy, 2018). Descriptors of these 12 herbal compounds were calculated using the PADEL-descriptor calculator before calculating their biological activity.

Molecular docking simulation studies

Molecular docking studies of the reported 12 herbal compounds from the plant *G. arborea* was performed against the Janus kinase 3 (JAK3) domains which is the

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key enzymes of the cytokines family recognized to play an indispensable role in the management of RA. PDB 3LXK from the RCSB protein data repository is used for docking simulations. These simulations were run in order to find out how stable the receptor is for ligand complexes and to learn more about how the legends interact with the receptor. Using the Auto Dock Vina software tool, all molecular docking simulations were carried out in accordance with the procedure outlined by Trott *et al.* (2010).

Animals

Male Wistar rats having weight in the range of 200-250g were used in the entire *in-vivo* experimentation. According to the criteria of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment, Government of India, New Delhi, all experiments were carried out only after receiving consent from the Institute Animals Ethics Committee. The animals were kept in pathogen-free environments with a 12-hour light-dark cycle, a room temperature of 25 ± 2°C, and a humidity of 55 ± 5%. Water and food were freely available to animals. Every effort was made to reduce the animals' suffering during the trial. **Plant collection and extraction**

Fresh leaves of the *G. arborea* were collected from the region of Hamirpur Himachal Pradesh, India in the month of August. Leaves were cleaned to remove traces of soil and other impurities, followed by shade drying for four weeks. Leaves were coarsely grounded before being subjected to extraction using water and ethanol solvents in the Soxhlet apparatus. The prepared extract was filtered through muslin cloth, concentrated in a rotary evaporator (Heidolph 4011, USA) till thick consistency was achieved, and then freeze-dried using lyophilizer to obtain a dried powder of the extract. The extractive yield of the aqueous and ethanol extract was found to be 14.6% and 17.2% respectively. The powdered extract was stored at 4°C until used for the experimentation in the desired dose.

Preparation of test samples and animals

Aqueous and ethanol extract of the leaves of *G. arborea* (AGA and EGA, respectively) were administered orally to the animals at a dose of 200 to 500 mg/kg, once daily. Extracts were prepared for dosing by suspending the required quantity in 0.5% w/ml cellulose gum in normal saline (vehicle). Extracts were prepared freshly before being administered to the animals. Animals were divided into five groups (n = 6) and were made familiar with the laboratory setting by placing their home cages in the experiment room 24 h before the start of experiments.

***In-vivo* anti-inflammatory activity (Carrageenan induced inflammation)**

Plant G. arborea's ethanol-based and aqueous extract's anti-inflammatory properties were assessed using the carrageenan-induced paw edoema test. The right hind paw's planar tissue was injected with 0.1 ml of carrageenan (1% solution in normal saline) to cause edoema in the female Wistar rats. Eight groups of six animals each were used to split the animals, and the percentage of each group that was protected against inflammation caused by carrageenan was noted (in comparison to control). Following were the animal groupings and treatments carried out.:

Group 1: Normal control (NC); received 0.5 ml vehicle orally once daily

Group 2: Standard; received 40 mg/kg indomethacin in vehicle orally 1 hour before injecting carrageenan

Group 3: AGA 200; received 200 mg/kg aqueous extract in vehicle orally 1 hour before injecting carrageenan

Group 4: AGA 400; received 400 mg/kg aqueous extract in vehicle orally 1 hour before injecting carrageenan

Group 5: AGA 500; received 500 mg/kg aqueous extract in vehicle orally 1 hour before injecting carrageenan

Group 6: EGA 200; received 200 mg/kg ethanol extract in vehicle orally 1 hour before injecting carrageenan

Group 7: EGA 400; received 400 mg/kg ethanol extract in vehicle orally 1 hour before injecting carrageenan

Group 8: EGA 500; received 500 mg/kg aqueous ethanol in vehicle orally 1 hour before injecting carrageenan

The paw volumes (ml) of all the treatment groups were measured after 04 hours of administration of the carrageenan by employing the mercury displacement technique using a Plethysmograph.

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Adjuvant-Induced Arthritis (AIA) in Rats

The AIA model was performed in rats to evaluate the effect of extract treatment on RA. Based on the results of the carrageenan-induced inflammation, a dose of 250 mg/kg for both plant extracts was selected for this study. Animals were grouped into 5 groups having 6 animals in each group.
Animal grouping and treatments are depicted below.

Group 1: Normal control (NC); received 0.5 ml vehicle orally once daily

Group 2: Disease controls (DC); received 0.5 ml vehicle orally once daily

Group 3: Standard (MTX10); received 10 mg/kg metho-trexate in vehicle orally once daily

Group 4: Aqueous extract; received 250 mg/kg aqueous extract in vehicle orally once daily

Group 5: Ethanol extract; received 250 mg/kg extract in vehicle orally once daily

Arthritis was induced by injecting 0.2 ml Complete Freund's adjuvant (CFA; containing 10 mg/ml of heat-killed *M. tuberculosis*) into groups 2-5. The standard drug, Metho-trexate, and different plant extracts were administered orally once daily as indicated above for 28 days. The animals of the normal control (NC) and disease control (DC) were administered 0.5 ml vehicle only daily.

Evaluation of Arthritis

Functional parameters

It is a well-established fact that RA is associated with the development of many visible impairments in the joint that progresses with time. These parameters can be used to evaluate the severity and progression of RA through tests like the Gait test, mobility test, joint stiffness, and visual weight-bearing test. In the present study, we investigated the effect of the extract treatment on the development and progression of RA by evaluating these parameters.

Arthritic index

Each animal was evaluated for the severity of arthritis on alternate days till the completion of the study. Each observation was marked by different researchers to eliminate any bias. In our study we used a widely accepted 5-point scoring scale to measure arthritis scores (Mehta *et al.*, 2023): normal paw = 0, minor inflammation and sign involving ankle/wrist = 1, inflammation and signs involving ankle and tarsal of the hind paw or/and wrist and carpals of fore paw = 2, severe inflammation and arthritic signs extended to metatarsals and metacarpals = 3, a severe disease involving entire hind and fore paw or/and subcutaneous arthritic nodules = 4. The maximum arthritic score was set at 16 per rat (4 points × 4 paws).

Hind paw swelling

The volume of hind paw swelling was measured by using Plethysmometer on 7, 14, 21, and 28 days of the study. The experiment was performed in triplicate and the mean value was taken as the final score.

Spleen index

On day 28, all rats were sacrificed by cervical dislocation, and their spleens were extracted. The spleen was weighed immediately after dissection and the spleen index was measured by using the following equation (Chen *et al.*, 2010).

$$\text{Spleen index} = \frac{\text{Spleen weight of sacrificed animal}}{\text{Body weight of sacrificed animal}}$$

Hematological Parameters

Blood samples from the retro-orbital plexus of the animals were collected for the evaluation of the hematological parameters immediately before sacrificing the animal. Red blood cell (RBC), White blood cell (WBC) platelet count and Erythrocyte Sedimentation Rate (ESR) were the parameters evaluated of the blood samples by using commercially available kits.

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Biochemical profile

Again for the study of biochemical profile, blood sample from the retro-orbital venous plexus of rats was collected on the last day serum was prepared and evaluated for Serum Glutamic Pyruvic Transaminase (SGPT), Serum Glutamic Oxaloacetic Transaminase (SGOT), and Alkaline Phosphatase (ALK) levels by using commercially available kits.

Statistical analysis

All the results are expressed as mean ± SD. One-way ANOVA followed by Dunnett's *t*-test using Graph-Pad Prism software was used to estimate the statistical significance of the results. Results were considered statistically significant at $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$.

RESULTS

QSAR analysis

Descriptor selection was done by employing the GA approach for the generation of MLR equation using 04 descriptors. Details of the descriptors used for the generation of the QSAR model are given in Table I.

The MLR QSAR equation generated is as follows:

$$\%\ inhibition = 42.7211 + 0.0013\text{ATSC3e} + 0.0124\ \text{GATS1p} - 0.0057\text{SpMax1\_Bhs} + 0.0120\text{PetitjeanNumber}$$

Where $n_{train} = 19$, $R^2_{train} = 0.8776$ $R^2_{adj} = 0.8426$, RMSE tr: 0.0002. MAE tr: 0.0002, $Q^2(LOO) = 0.7849$, $n_{test} = 7$, $R^2_{test} = 0.83852$ and (MAE; 95% data): MAE tr: 0.0002.

From the above model, ATSC3e, GATS1p, and Petit jean Number were observed to be the most significant descriptors with positive contributions, along with SpMax1_Bhs that was having negative contribution in the developed model. The values of R^2_{train} and R^2_{test} in the developed model were observed to be 0.8776 and 0.83852, respectively. These values confirmed that there is a good extrapolation between the training and test dataset. The robustness of the generated QSAR model was validated further by a small difference between R^2_{train} and Q^2 (<0.5%). The plot between predicted PIC50 and observed PIC50 is given in Figure 1.

Virtual screening and docking analysis

The predicted percentage inhibition of the 12 herbal compounds reported in the *G. arborea* is given in Table II. Our results predicted luteolin, apigenin, campesterol, and stigmasterol to have good biological activity with scores of 70.30, 70.43, 75.14, and 78.52, respectively. Other bioactive molecules also demonstrated a biological activity score in the range of 52.02 to 64.98, which were not as good as the above molecules.

To further validate the results of the generated 2D-QSAR model, we subjected these bioactive molecules to molecular docking screening by using Auto Dock Vina against the Janus kinase 3 (JAK3) target of RA. The results of the molecular docking were expressed as dock score (Kcal/mol), number of hydrogen bonds formed between the protein and ligand, amino acid residues involved, and bond length (Table III).

According to the molecular docking study's findings, every bioactive compound in G. arborea has a docking score higher than the internal benchmark, 3 (R, 4).methyl(7H-pyrrolo[2,3-d]pyrimidin4-yl)amino]piperidin-1-yl}-4-methyl-3-[methyl-3-oxopropanenitrile, which had a -6.6 Kcal/mol docking score. These findings imply that the existence of these bioactive compounds may be responsible for G. arborea's anti-inflammatory action and positive effects during RA. The most promising bioactive compounds of G. arborea were predicted by combining the findings of the 2D-QSAR and molecular docking study. These compounds showed good biological activity in the created 2D-QSAR model and had docking scores of -7.6, -7.2, -7.7, and -7.4 respectively, which was better than the internal standard. The compounds were identified as luteolin, apigenin, campesterol, and stigmasterol. Figure 2 shows the pictures of these compounds' molecular docking interaction.

***In-vivo* anti-inflammatory activity (Carrageenan-induced rat paws edema)**

The effect of the ethanol (EGA) and aqueous (AGA) extract of the *G. arborea* on the inflammation was evaluated through carrageenan-induced rat paw edema at a dose of 200, 400, and 500 mg/kg, and the results were compared to indomethacin (used as a standard drug). The

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results are depicted as percent inhibition of paw edema (inflammation) after 4 h of carrageenan injection in Figure 3.

Evaluation of Arthritis

Functional parameters

RA is associated with the development of much visible impairment in the joints, This may be assessed using the visual weight-bearing test, joint stiffness, gait test, and mobility test. These parameters can be used to evaluate the severity and progression of RA and we investigated the effect of the extract treatment on the development and progression of RA by evaluating these tests. The results are depicted in Table IV.

Injecting CFA into the rats resulted in significant ($p < 0.05$) impairments in the animal gait, mobility, and visual weight bearing, besides, resulting in significant ($p < 0.05$) joint stiffness. These animals appeared to stand and walk with visible signs of pain and joint stiffness, suggesting the progression and development of RA. Treating these animals with the ethanol and aqueous extract of *G. arborea* resulted in a significant ($p < 0.05$) improvement in all these parameters and the results were comparable to metho-trexate. Although the results of both extract treatments were comparable to the standard, the effect of the aqueous extract seems to be much better than the ethanol extract. These results suggest that extract treatment is having a good potential to provide symptomatic relief and protection against the development and progression of RA.

Arthritis Index:

The arthritis index serves as a quantitative measure of disease severity. It considers parameters like joint inflammation, swelling, and mobility impairment. This index aids researchers in evaluating disease progression and the effect of various treatments. The progression of RA in the rats after CFA injection was observed for 28 days by different researchers and the results are depicted in Figure 4. Our results suggest that CFA injection resulted in significant ($p < 0.001$) development in the RA as indicated by the continuous increase in the arthritis index over the period of 28 days. Treating these animals with the ethanol and aqueous extract of *G. arborea* resulted in a significant ($p < 0.001$) improvement in the arthritis index which was lower than the DC throughout the study. These results were comparable to metho-trexate, which also demonstrated a significant ($p < 0.001$) improvement in the arthritis index during the study. Although the results of both extract treatments were comparable to the standard, the effect of the aqueous extract seems to be much better than the ethanol extract. These results suggest that extract treatment is having a good potential to retard the progression of RA.

Spleen Index:

Studying the spleen index in an animal model of arthritis can provide valuable insights into the immune response and inflammatory processes associated with arthritis. The spleen is a critical organ in the immune system, and changes in its size and function often reflect the systemic immune response to various stimuli, including inflammatory conditions like arthritis. We evaluated the effect of extract treatment on the spleen index as a marker of RA severity and progression and the results are depicted in Figure 5. Our results suggest that the spleen index of DC rats was significantly ($p< 0.001$) high when compared to NC animals. Treating these animals with the ethanol and aqueous extract of *G. arborea* resulted in a significant ($p < 0.001$) improvement in the spleen index in treated animals. These results were comparable to the standard drug, metho-trexate, which also demonstrated a significant ($p< 0.001$) improvement in the spleen index. Although the results of both extract treatments were comparable to the standard, the effect of the aqueous extract seems to be much better than the ethanol extract.

Hematological Parameters

In animal models of arthritis, which are used to simulate and study human RA, several of the levels of RBC, WBC, and ESR are the major hematological parameters that can be altered. Alterations in these parameters can be correlated to the stage of RA progression. We evaluated the effect of extract treatments on the blood levels of RBC, WBC, and ESR as the marker of the severity and progression of RA and the results are depicted in Table V.

During RA, the levels of ESR, polymorphonuclear leukocytes, and inflammatory monocytes are generally increased and the levels of RBCs, lymphocytes,

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granulocytes, and acidophils are decreased, which can be attributed to the development of inflammatory response in the body during RA. In our study, similar trends were observed in animals injected with CFA (DC). Both extract and metho-trexate, improved the altered levels of hematological parameters suggesting their potential to impart protection against inflammation during RA, however, no significant difference in the results was observed.

Biochemical profile

We investigated the effect of extract treatment on the serum levels of SGOT, SGPT, and ALP, which are considered to be prominent markers of the development and progression of RA. The results are depicted in Table VI.

It is a well-established fact that RA is associated with enhanced levels of serum SGOT, SGPT, and ALP. In our study, we also observed a significant ($p < 0.001$) increase in the serum levels of SGOT, SGPT, and ALP after CFA injection to the animals that confirmed the development of RA in these animals. Treating these animals with the ethanol and aqueous extract of *G. arborea* resulted in a significant reduction in the serum levels of SGOT, SGPT, and ALP that suggest that extract treatment s having the potential to alleviate complications associated with the RA. These results were comparable to metho-trexate treatment ($p < 0.001$) and the aqueous extract was observed to be slightly more efficient than ethanol extract when compared to control and standard.

DISCUSSION

Using the % inhibition of rat paw edema by 26 herbal compounds as a guide for model creation, we created a strong 2D-QSAR model in the current work to predict the anti-inflammatory capability of the herbal molecules. The choice of descriptors, which are intimately connected with the structural features of the molecules, is the primary factor in the building of a strong, effective, and statistically significant QSAR model (Tropsha, 2007). The descriptors for the current study were identified using PADEL software, yielding a total of 1875 descriptors. Additional software utilised for data pre-treatment and normalisation was DTC-QSAR v 1.0.6. During the data preparation procedure, the constant and associated descriptors with square correlation coefficient values greater than 0.85 were removed. Additionally, the pretreatment data was split into training sets (consisting of 70% of compounds) and test sets (30% of compounds) using the Kennard-Stone method. Table VII shows the acceptance criteria that Golbraikh and Tropsha (2002) suggested. The Golbraikh and Tropsha requirements of QSAR model robustness were effectively met by our model, as shown by the acceptance criteria and our findings. With success, we created a reliable 2D-QSAR model to estimate the herbal compounds' capacity to reduce inflammation. Our findings concur with other papers (Golbraikh, Tropsha, 2002) that used the Golbraikh and Tropsha criteria for the development and validation of QSAR models.

The constructed QSAR model's regression statistics are shown in Table VIII as p-values and tvalues. Given that the descriptor coefficients were shown to be statistically significant at a 95% confidence range, our results are consistent with prior studies. According to earlier studies, there shouldn't be a link between the chosen descriptors and particular targets in order to create a reliable and effective QSAR model (Verma et al., 2017). Table 9 shows the correlation matrix of the descriptors that were utilised to create the QSAR model in our investigation. The chart illustrates that the four descriptors we used for our study's model generation did not correlate, which led to the creation of a reliable and effective QSAR model. Moreover, Table IX's correlation matrix of the descriptors verified that there is no association among the descriptors that were utilized to create the QSAR model. Williams plot shown in Figure 07 clearly shows that training and test datasets employed for the QSAR model development are well within the AD of the model.

In order to create a QSAR model that could forecast the anti-inflammatory properties of the bioactive compounds found in G. arborea, a total of 26 herbal compounds were employed. Based on our findings, the most likely compounds that G. arborea may contain that have antiinflammatory properties are luteolin, apigenin, stigmasterol, and campesterol. Furthermore, these compounds may have a potential anti-inflammatory effect, according to the findings of the molecular docking research conducted against the Janus kinase 3 (JAK3) domains. These results are equally supported by the previous reports where luteolin, apigenin, campesterol,

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and stigmasterol have been demonstrated to possess good anti-inflammatory (Ginwala *et al*., 2019; Morgan *et al.*, 2021; Caporali *et al.,* 2022; Nazir *et al.*, 2023) and anti-arthritic activity (Maslikah *et al.*, 2019; Khan *et al.*, 2020; Shen *et al.*, 2020; Nazir *et al.*, 2023). These reports provide strong validation of our QSAR model and the results of the molecular docking studies.

An autoimmune disease that mostly affects the joints, RA is a chronic condition. Inflammation of the tissue lining the interior of the joints, known as the stratum synovial, is its defining feature. This inflammation can lead to joint pain, swelling, stiffness, and eventually joint damage if not adequately managed (Belaso *et al*., 2019; Mehta *et al*., 2023). In the present study, we use CFAinduced arthritis models in female rats. The female rats were considered to be used specifically in the study considering the fact that the female population is having a higher risk of RA than males. One of the most popular, trustworthy, and accepted models for examining how plant extract affects the onset and course of RA is the CFA-induced arthritis model. In inflammatory cell infiltration, synovial inflammation and loss of joint function, proliferation of synovial tissue, and aberrant gait, CFA-induced RA in mice mimics the clinical manifestation of the illness (Zhu et al. 2020; Ismail et al., 2022).

G. arborea has been traditionally employed in herbal medicine systems for managing rheumatoid arthritis. Its active compounds are believed to alleviate joint pain and inflammation associated with rheumatoid arthritis. While anecdotal evidence supports its historical use, scientific research on *G. arborea*'s effectiveness in treating rheumatoid arthritis is limited. Its anti-inflammatory and analgesic properties have made it a potential remedy. Folk medicine in India employs *G. arborea* extracts to manage joint pain and inflammation (Warrier *et al*., 2021). Studies highlight its potential to inhibit pro-inflammatory cytokines and modulate immune responses (Khadanga, Nayak, 2017). Using the CFA model in rats, we assessed the anti-arthritic potential of ethanol and aqueous extract G. arborea in the current study. Our findings indicate that G. arborea may be able to attenuate RA, which offers compelling experimental support for its traditional usage.

Anti-inflammatory effects of the ethanol and aqueous leaf extract of *G. arborea* was evaluated through the rat paw edema method using a plethysmometer at the oral dose of 200, 400, and 500 mg/kg after 4 hours of carrageenan injection. Both the extracts demonstrated significant inhibition of inflammation when compared to the results standard drug, indomethacin. These findings are in agreement with the previous reports where *G. arborea* has been demonstrated to have the potential to reduce inflammation (Wadasinghe *et al.*, 2022). Based on the outcome of the anti-inflammatory activity, a dose of 250 mg/kg was selected for the further investigation of the anti-arthritic potential of *G. arborea* by using CFA induced RA model in female rats.

The severity and progression of arthritis in rats were evaluated throughout the study by evaluating the arthritis index. In animal models of RA, an "arthritis score" is a crucial tool for assessing disease severity and tracking the progression of the condition. It provides a quantifiable way to measure the degree of joint inflammation, swelling, and other relevant symptoms associated with RA (Mehta *et al.*, 2023). Animals in the DC group demonstrated a continuous increase in the arthritis index indicating the severity and progression of RA. Both the extract treatments significantly reduced the arthritis score and demonstrated promising results that were comparable to metho-trexate. Moreover, these results were further confirmed by evaluating the effects of extract treatments on the spleen index, hematological parameters, and serum levels of SGOT, SGPT, and ALP. RA is associated with the development of much visible impairment in the joints, which can be evaluated through the Gait test, mobility test, joint stiffness, and visual weightbearing test. These parameters can be used to evaluate the severity and progression of RA. We observed that CFA injection was associated with clear signs of development of severe RA. Studying the spleen index and hematological profile in an animal model of arthritis can provide valuable insights into the immune response and inflammatory processes associated with arthritis (Mehta *et al.*, 2023). The spleen is a critical organ in the immune system, and changes in its size and function often reflect the systemic immune response to various stimuli, including inflammatory conditions like arthritis (Mehta *et al.*, 2023). The spleen index was observed to be significantly higher in the DC group along

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with elevated levels of SGOT, SGPT, ALP, polymorphonuclear leukocytes, and macrophage and reduced levels of RBCs, lymphocytes, granulocytes, and acidophils. All these observations are reported in the literature to be associated with the development and progression of RA (Köhler *et al.* 2019; Pourhabibi Zarandi *et al.* 2021; Mehta *et al.*, 2023). Treating CFA animals with plant extract resulted in significant improvement in all these parameters, suggesting the good potential of plant extract to attenuate the physiological changes associated with RA to provide relief from the disease progression and symptoms. These findings are in line with the previous reports where research into the use of *G. arborea* in animal models of arthritis suggests potential anti-inflammatory and analgesic properties. A study by Khadanga and Nayak (2019) explored its effects in complete Freund's adjuvant-induced arthritis in rats. They found that Gmelina arborea extract significantly reduced paw edema, inflammation, and pain. Another study by Kumar *et al*., (2014) investigated its anti-arthritic potential in adjuvantinduced arthritis, highlighting its ability to suppress inflammatory markers and protect joint tissues. Although both extracts demonstrated good potential against CFA-induced RA in rats, the results of the aqueous extract were better than the ethanol extract in every observation. Our findings provide a strong justification for the traditional use of *G. arborea* during RA and inflammation.

CONCLUSION

The current study has been performed with the aim to provide an experimental and scientific justification for the traditional use of *G. arborea* during inflammation and RA. In this context, we successfully developed a robust and efficient QSAR model which can be used to predict the antiinflammatory activity of the herbal molecules. Results of the QSAR and molecular docking study predicted luteolin, apigenin, campesterol, and stigmasterol to be the most promising molecules that could be responsible for the traditional anti-inflammatory and anti-arthritic potential of *G. arborea*. Animal experimentation suggests that both studied extracts are having good potential against RA in rats, however, the aqueous extract is having a better effect on RA. Our findings provide a strong justification for the traditional use of *G. arborea* during RA and inflammation and we suggest that the anti-arthritic potential of *G. arborea* could be attributed to the presence of luteolin, apigenin, campesterol, and stigmasterol in the plant.

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AUTHOR CONTRIBUTION

MLK contributed to the study design, statistics of the data, and manuscript editing. PD performed all the experiments, collected data, and analyzed it, along with preparing the manuscript draft.
MSA contributed to study design, data analysis, and manuscript editing, and finalizing the draft.

CONFLICT OF INTEREST

Authors declare no conflict of interest of any kind related to this work

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List of Figures:

Figure 1: The plot between predicted PIC50 and observed PIC50.

Figure 2: The images of the molecular docking interaction of internal standard, luteolin, apigenin, campesterol, and stigmasterol with Janus Kinase 3 (PDB ID: 3LXK).

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Figure 3: Effect of EGA and AGA on rat paw edema after 4 h of carrageenan injection. EGA 200 = 200 mg/kg ethanol extract of G. arborea; EGA 400 = 400 mg/kg ethanol extract of G. arborea; EGA 500 = 500 mg/kg ethanol extract of G. arborea; AGA 200 = 200 mg/kg aqueous extract of G. arborea; AGA 400 = 400 mg/kg aqueous extract of G. arborea; AGA 500 = 500 mg/kg aqueous extract of G. arborea; Indomethacin 40 = 40 mg/kg dose of indomethacin. Values are represented as mean ± SD. The significance of the results are depicted as $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ compared to standard.

Figure 4: Effect of EGA and AGA on arthritis index during 28 days of study. NC = Normal control. DC = Disease control. EGA 250 = 250 mg/kg ethanol extract of G. arborea; AGA 250 = 250 mg/kg aqueous extract of G. arborea; MTX 10 = 10 mg/kg dose of methotrexate. Values are represented as mean ± SD. The significance of the results are depicted as $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ (compared to NC) and $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, and $^{\#\#\#}p < 0.001$ (compared to DC).

Figure 5: Effect of EGA and AGA on spleen index after 28 days of CFA injection. NC = Normal control. DC = Disease control. EGA 250 = 250 mg/kg ethanol extract of G. arborea; AGA 250 = 250 mg/kg aqueous e xtract of G. arborea; MTX 10 = 10 mg/kg dose of methotrexate. Values are represented as mean ± SD. The significance of the results are depicted as *p < 0.05, **p < 0.01, and ***p < 0.001 (compared to NC) and $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, and $^{\#\#\#}p < 0.001$ (compared to DC).

Figure 6: The scatter plot of standardized residuals and leverages (Williams plot)

Table I: Details of the descriptors employed for the generation of the QSAR model

S. No.	Descriptor	Description	Class	Type	Contribution
1	ATSC3e	Weighted by Sanderson electronegativities and centered Broto-Moreau autocorrelation with latency of three	Auto correlation	2D	Positive
2	GATS1p	Geary autocorrelation of lag 1 polarizability-weighted	Auto correlation	2D	Positive
3	SpMax1_Bhs	n 1 / weighted by relative Istate represents the greatest absolute eigenvalue of the Burden modified matrix.	Burden Modified Eigenvalues descriptor	2D	Negative
4	Petitjean Number	A molecular graph topology characterization index.	Auto correlation	2D	Positive

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Table II: Predicted biological activity (% inhibition) of the 12 bioactive molecules of *G. arborea*

S. No.	Name of compound	Predicted % inhibition (Biological Activity)
1.	Apigenin	70.43
2.	Arboreal	59.63
3.	Balanophonin	64.98
4.	Campesterol	75.14
5.	Gmelanone	55.26
6.	Gummadiol	52.02
7.	Isoarboreal	56.56
8.	Kaempferol	56.15
9.	Lignansarborone	63.76
10.	Luteolin	70.30
11.	Quercetin	56.15
12.	Stigmasterol	78.52

Table III: Dock score of the compounds from *Gmelina arborea* with the Janus kinase 3 (JAK3) domains along with interactions

S. No.	Name of compound	Dock Score (KCal/mol)	No. of H-Bond	Amino acid Residues	Bond Length (A^0)
1	3-{(3R,4R)-4-methyl-3(7H-pyrrolo[2,3d]pyrimidin-4yl)amino]piperidin-1-yl}-3oxopropanenitrile	-6.6	01	Gln988	2.120
2	Apigenin	-7.2	02	Val983 Gln988	2.214 1.985

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3	Arboreal	-8.2	02	Val983 Gln988	2.113 2.055
4	Balanophonin	-6.9	02	Ser1005 Ser1005	2.991 2.970
5	Campesterol	-7.7	01	Val983	2.146
6	Gmelanone	-8.0	01	Gln1083	2.097
7	Gummadiol	-7.5	00	----	----
8	Isoarboreal	-7.8	01	Arg948	2.125
9	Kampferol	-7.1	00	----	----
10	Lignansorborone	-7.8	01	Leu970	3.094
11	Luteolin	-7.6	04	Arg948 Gln988 Glu985 Arg946	2.145 1.820 2.213 2.149
12	Quercetin	-7.2	02	Val983 Gln988	2.064 1.853
13	Stigmasterol	-7.4	00	----	----

Table IV: The impact of G. arborea ethanol and aqueous extract on the functional indices of rats with CFA-induced arthritis. NC stands for normal control. DC stands for disease control. AGA 250 = 250 mg/kg aqueous extract of G. arborea; MTX 10 = 10 mg/kg dosage of methotrexate; EGA 250 = 250 mg/kg ethanol extract of G. arborea. The values are shown as mean ± SD. *p < 0.05, **p < 0.01, and ***p < 0.001 (in comparison to NC) and #p < 0.05, ##p < 0.01, and ###p < 0.001 (in comparison to DC) indicate the importance of the findings.

Groups	**Gait test**	**Mobility test**	**Joint stiffness**	**Visual weightbearing test**
NC	0 ± 0.00	8.65 ± 0.10	0 ± 0.00	0 ± 0.00
DC	2.54 ± 0.65*	4.10 ± 0.75*	1.25 ± 1.40*	1.45 ± 0.30*
MTX10	0.18 ± 0.02$^{\#}$	5.65 ± 2.32$^{\#}$	0.40 ± 0.35$^{\#}$	0.00 ± 0.01$^{\#}$
EGA 250	0.25 ± 0.10$^{\#}$	7.90 ± 2.70$^{\#}$	0.60 ± 0.55$^{\#}$	0.20 ± 0.31$^{\#}$

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AGE 250	0.19 ± 0.20$^{\#}$	5.15 ± 2.20$^{\#}$	0.40 ± 1.00$^{\#}$	0.14 ± 1.09$^{\#}$

Table V: The impact of ethanol and G. arborea aqueous extract on changes in haematological parameters. NC stands for normal control. DC stands for disease control. G. arborea ethanol extract (EGA 250 = 250 mg/kg), aqueous extract (AGA 250 = 250 mg/kg), and metho-trexate (10 mg/kg) dosage (MTX 10 = 10 mg/kg). The values are shown as mean ± SD. *p < 0.05, **p < 0.01, and ***p < 0.001 (in comparison to NC) and #p < 0.05, ##p < 0.01, and ###p < 0.001 (in comparison to DC) indicate the importance of the findings.

Parameters	**NC**	**DC**	**MTX 10**	**EGA 250**	**AGA 250**
RBC (10^6/mm^3)	6.78±1.75	6.30±1.24	6.85±1.14	6.80±1.22	6.90±1.80
ESR (mm/hr)	5.68±1.32	7.34±0.15	7.15±1.45	7.40±1.76	7.50±1.15
Lymphocytes (%)	55.30±5.95	54.00±4.15	53.00±5.65	54.00±6.70	53.00±5.70
Neutrophils (%)	37.75±0.68	39.25±4.90	38.80±6.20	38.40±5.60	37.50±6.54
Monocytes (%)	4.00±0.50	5.00±0.10	4.21±0.20	5.00±0.85	4.90±0.70
Basophils (%)	0.07±1.15	0.03±0.01	0.06±0.08	0.05±0.02	0.05±0.08
Eosinophils (%)	1.12±0.09	1.09±0.16	1.04±0.65	1.13±0.34	1.18±0.50

Table VI: The impact of ethanol and G. arborea aqueous extract on changes in SGOT, SGPT, and ALP serum levels. NC stands for normal control. DC stands for disease control. MTX 10 is a dosage of methotrexate equivalent to 10 mg/kg. EGA 250 is an ethanol extract of G. arborea; AGA 250 is an aqueous extract of G. arborea. The values are shown as mean ± SD. *p < 0.05, **p < 0.01, and ***p < 0.001 (in comparison to NC) and #p < 0.05, ##p < 0.01, and ###p < 0.001 (in comparison to DC) indicate the importance of the findings.

Groups	**SGOT (IU/L)**	**SGPT (IU/L)**	**ALP (IU/L)**
NC	158 ± 10.15	170 ± 13.75	195 ± 10.60
DC	280 ± 20.75***	320 ± 18.24***	354 ± 15.14***
MTX 10	220 ± 14.55$^{\#\#\#}$	249 ± 14.56$^{\#\#\#}$	278 ± 12.70$^{\#\#\#}$
EGA 250	190 ± 20.67$^{\#\#\#}$	170 ± 10.70$^{\#\#\#}$	160 ± 15.45$^{\#\#\#}$
AGA 250	171 ± 15.70$^{\#\#\#}$	164 ± 10.00$^{\#\#\#}$	155 ± 10.15$^{\#\#\#}$

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Table VII: Result of the Golbraikh and Tropsha parameters of the developed QSAR model

S. No.	Parameter	Threshold value	Model Score
1	Q^2	Threshold value $Q^2 > 0.5$	0.7849
2	R2test	Threshold value $R^2_{test} > 0.6$	0.83852
3	|R20-R'20|	Threshold value $\|r_0^2 - r'^2_0\| < 0.3$	0.0350
4	k or k'	$0.85 < k < 1.15$ and $0.85 < k' < 1.15$	1 or 1.000
5	R2test-R20/R2test	Threshold value $R^2 - R^2_0/R^2 < 0.1$	0.04138

Table VIII: The p and t value of regression statistics of the developed QSAR model

	Coefficients	Standard Error	t Stat	P-value
Intercept	42.7211	0.0079	0.0169	0.0000
ATSC3e	0.0013	0.0003	0.005	0.0001
SpMax4_Bhm	0.0124	0.0017	0.0037	0.0000
SpMax1_Bhs	-0.0057	0.0014	0.0030	0.0010
Petitjean Number	0.0120	0.0038	0.0081	0.0052

Table IX: Correlation between the selected descriptors for the development of the QSAR model

	ATSC3e	SpMax4_Bhm	ATSC1e	GATS5v
ATSC3e	1			
GATS1p	-0.0463	1		
SpMax1_Bhs	-0.0945	-0.0001	1	
Petitjean Number	0.2044	-0.2054	-0.3700	1

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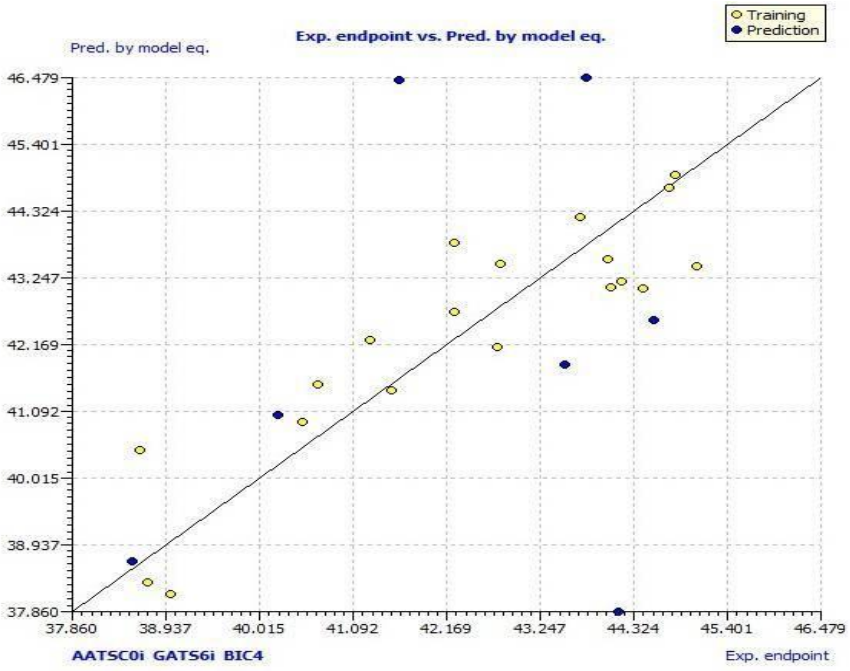

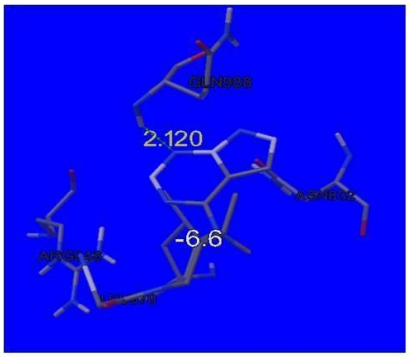

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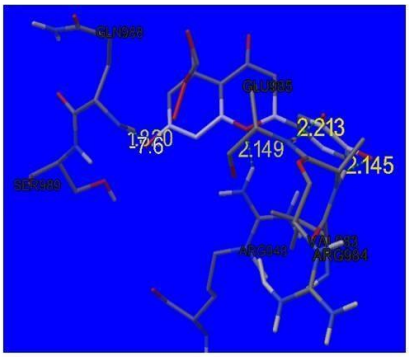
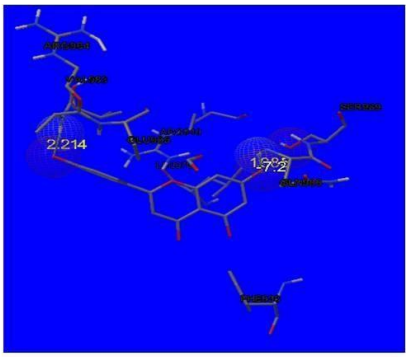
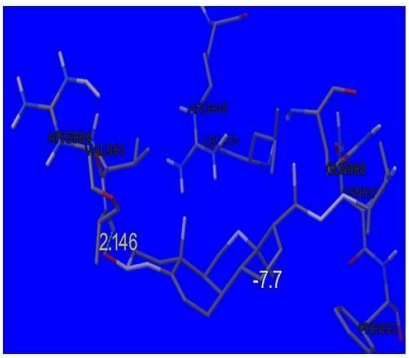


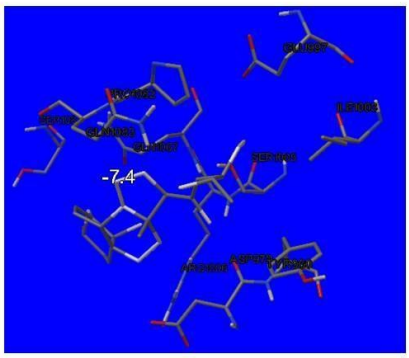
Luteolin



Apigenin



Campesterol



Stigmasterol

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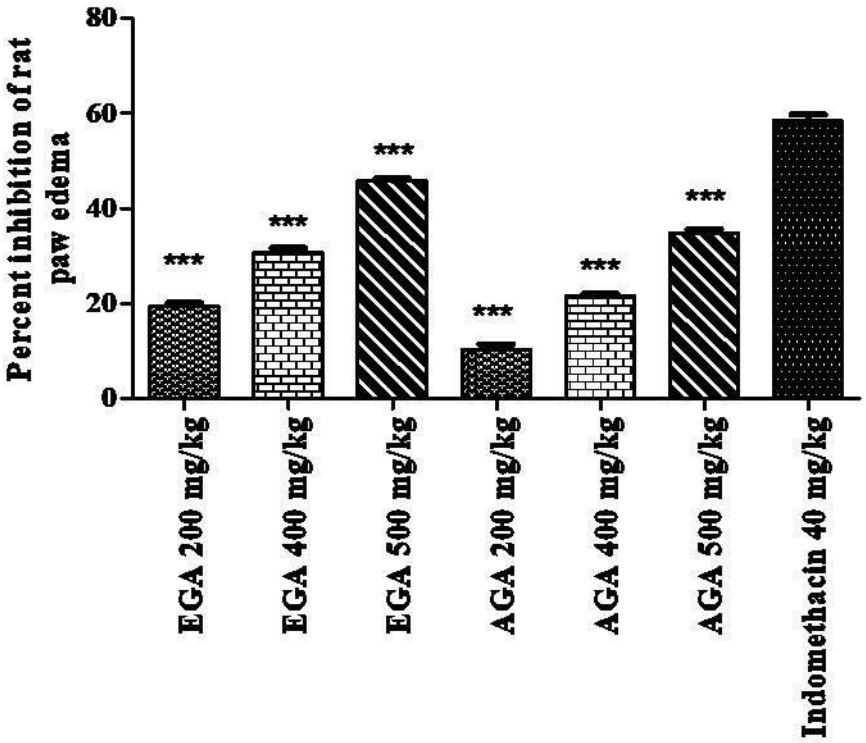

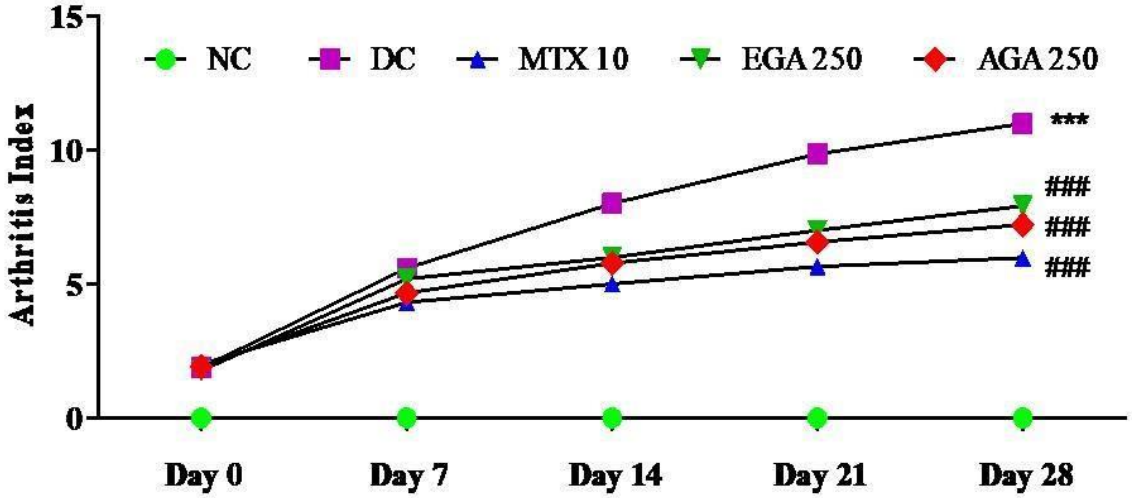

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