# Molecular docking and *in vitro* studies of *Malva Sylvestris* for Anti-arthritic, Anti-diabetic, Anti-coagulant, Anthelmintic and Antioxidant activity

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# Abstract

*Malva sylvestris L.,* known by the name mallow is mostly used in traditional system of medicine owing to its miraculous therapeutic potentials. Due to its wide therapeutic applications many research work has been conducted and in this regard an attempt has been made to evaluate the pharmacological efficacy of *Malva sylvestris* as a potent anticoagulant, anthelmintic, anti-diabetic, antioxidant and anti-arthritic agent by *in-vitro* methods. To validate the research work, the active constituents (Quercetin, Apigenin, Kaempferol and Malvidin) are evaluated for the activities via molecular docking studies using software PyRx. *In vitro* biological efficacy of *Malva sylvestris* fractional extract (ethanolic, hexane and chloroform) was evaluated for anti-arthritic, anticoagulant, anthelmintic, anti-diabetic and antioxidant activity by means of egg albumin denaturation procedure, Prothrombin time, Paralysis and death time, α-amylase inhibitory activity and DPPH free radical scavenging potential. Out of all the extract fractions, the ethanolic extract has demonstrated promising therapeutic potential in all the activities at different concentrations (100, 300, 500 µg/ml) with $IC_{50}$ value within 59.33±10.26-154.41±11.16 µg/ml range. The experimental statistics offers insight into the potential of the plants in numerous novel target-based therapies which supports the compounds medicinal potential and implicates the therapeutic efficacy of the plant as potential source of medicament.


**Keywords:** *Malva sylvestris*, Molecular docking, Anticoagulant, Anthelmintic, Anti-diabetic, Antioxidant, Anti-arthritic

# 1.INTRODUCTION

For millennia, traditional herbs were the only available convenient sources for numerous ethnic groups to cure, alleviate or prevent a wide range of health disorders[1]. Amidst the diverse herbs utilized as therapeutic source in herbal system, *M. sylvestris* is a remarkable edible plant owing to its extensive therapeutic potentials, with its utilization reported to have emerged in 3000 BC[3]. The plant preferably grows in clammy place nearby the salty wetland, ocean, pastureland, tidal river banks and encompassing the ditches[4]. *Malva sylvestris* is a biennial–perennial herb consists of a juicy annual stem with a height of 2-3 feet and has a perennial root having leaves which are with purplish flowers which generally bloom in late spring season. This Leaves are soft, shallowly lobed, large, broadly heart-shaped and plaited. Flowers are purplish coloured, odourless which closely resemble that of honeysuckle[5]. The entire plant is reported to possess therapeutic activities but the potential pharmacological actions of the herb is mainly concentrated to the flowers and leaves due to some mucilage and flavonoids which are mostly accumulated in those parts[6-17]. In the leaves and flowers, various flavonoid and anthocyanin contents are endowed such as Quercetin, Apigenin, Kaempferol and Malvidin which deploys a myriad of potential effects on human health. Malva sylvestris have some of the most remarkable health benefits including the capacity to promote healing of wounds, prevent infection, lower inflammation, lessen ageing symptoms, enhance respiratory health, optimise digestive processes, enhance sleep and alleviate headaches[17-27]. The inflorescence is employed for treating inflammation, cough, eczema, wounds, bronchitis, antimicrobial, anticancer, sore throat, skin whitening, dermal infected wounds alopecia and digestive problems[28]. Anthocyanins from *Malva sylvestris* are reported to lower the total cholesterol and triglycerides level of plasma. The malva extracts exhibit potent gastroprotective/antiulcerogenic activity due to the presence of high mucilage content[29-33]. This research work emphasizes on evaluation of anticoagulant, anthelmintic, anti-diabetic, antioxidant & anti-arthritic activity of ethanolic, chloroform and hexane extracts of plants by *in-vitro* methods with a prior exploration of docking analysis.

# 2. MATERIALS AND METHODS

**2.1. Molecular Docking:** Software PyRx was employed for Molecular docking of Quercetin, Apigenin, kaempferol and Malvidin. The target proteins with PDB ID: 3CTT (anti-diabetic), 4Z69(anti-arthritic), 2CDU (antioxidant), 4MS3(anthelmintic) & 2GDE (anticoagulant) were obtained from RCSB Protein Data Bank. Biovia Discovery Studio was used for the preparation of crystal structure of proteins by removing water molecules, heteroatoms and addition of polar hydrogen atoms. The structure of the ligands was prepared using Chemsketch and the energy of the ligands were minimized using Chem 3D software.

**2.2. *In-vitro* activity**

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**2.2.1. Antioxidant activity (DPPH method)**: DPPH antioxidant reagent (0.13 mM) solution and various plant fractions were mixed with 1 ml of ethanol, swirled and allowed to fully mix. A UV spectrophotometer at 490 nm measured absorbance after the reaction mixture was let to stay at room temperature (25 $^0$C) in the dark for 15 to 20 minutes. DPPH's capacity to scavenge radicals is determined by the formula below[34]:

Percentage inhibition = $[(A_0 - A_1)/A_0 \times 100]$, where $A_0$ & $A_1$ is the absorbance of the blank sample & extract solution respectively.

**2.2.2. Anti-arthritic activity (Egg Albumin Denaturation method):** Various fractionated extracts were mixed with egg albumin (0.2 mL) and $Na_3PO_4$ buffered saline (2.8 mL, pH 6.4). The final sample solution contents were then mixed with distilled water and the solution was made up to 5 mL. Diclofenac along with a double-distilled water was utilised as the standard and the control solution respectively. The fractionated extracts were heated to 70 °C for approximately 5 minutes and it was placed in a biological oxygen demand (BOD) chamber and incubated (37 °C, 15 mins). Following cooling of the solutions, the absorbance was measured at 660 nm[35]. The following formula determines the denaturation of the egg albumin protein:

Percentage inhibition = $[(A_0 - A_1)/A_0 \times 100]$, where $A_0$ & $A_1$ is the absorbance of the blank sample & extract solution respectively.

**2.2.3. Anticoagulant activity (Prothrombin time method):** To stop the blood clotting process, the extracted materials were put in a beaker with a 3.8% solution of trisodium citrate. Centrifugation was used at 3000 rpm speed for a time interval of 15 minutes to separate the plasma from the blood cells which resulted in the formation of pure plasma containing platelets needed for the activity. Following centrifugation, the following ingredients were added to a fusion tube: plasma 0.2 ml, methanolic extract solution of 0.1 ml containing 100, 300, and 500 mg/ml of *M. sylvestris* and 0.3 ml $CaCl_2$. The tube containing all the contents was heated at 37$^0$C in a water bath. The standard drug used for the experiment was warfarin and 0.9% saline water dissolved in methanolic solution served as a control. Using a stopwatch, the blood's clotting time was determined by tilting[36].

**2.2.4. Anthelmintic activity (Paralysis & Death time):** Earthworms were extracted from damp soil and cleaned using regular saline water. Because earthworms and human intestinal roundworm parasites are similar in anatomy and physiology, earthworms were used in this investigation. Before the experiment began, a fresh 5% Dimethyl sulfoxide (DMF) solution was used to prepare all of the test solutions as well as the reference drug solution. In petri dishes, 5% DMF solution was poured and 6 groups of roughly equal-sized earthworms were placed and then 25 ml solutions containing 25, 50 and 100 mg/ml concentrations of *M. sylvestris* was used. In this study, albendazole served as the reference medication. The paralysis and death time taken by each worm were recorded. When all the worms were vigorously shaken and no movement was seen, paralysis of the worms took place. After determining that the worms skin colour was gradually fading away and the worms remained motionless when shaken hard or upon submerging in water at a temperature of 50 °C, the duration by the worms to get paralysed and die was recorded[37].

**2.2.5. Antidiabetic activity (α-amylase inhibitory activity):** Stock solution was created by dissolving 100, 300 and 500µg/ml concentration of *M. sylvestris* in 1 ml dimethyl sulfoxide (DMSO) solution. 250µL of stock solution of *M. sylvestris* was added to a tube possessing 25 µL α-amylase and sodium phosphate buffer (250µL, 0.02M, pH 6.9). Pre-incubation was done for 10 minutes 28 ± 2 $^0$C temperature. To the mixture, 1% solution of starch of concentration 250µL and $Na_3PO_4$ buffer (0.02M, pH 6.9) was poured and the resultant mixture was set for incubation for an additional 10 minutes at 25°C. Quenching of the resultant solution was done by adding yellow-orange coloured DNSA/Dinitro Salicylic Acid (400 µL) reagent. Test tubes containing all the ingredients were boiled for 3-5 minutes until a yellowish orange hue formed and it was cooled to 25 $^0$C following which distilled water of 5ml was mixed and using UV-visible spectrophotometer at 540 nm the extract's absorbance was noted down. The usual α-amylase inhibitor medication used was Acarbose and the blank solution was made in the same way by substituting distilled water for the test solution. Each test solution's α-amylase inhibitory activity is given as a percent inhibition, which can be computed as follows[38]:

Percentage Inhibition = $[(Absorbance_{control}\text{-}Absorbance_{extract})/Absorbance_{control}] \times 100$

# 3. RESULTS AND DISCUSSION

**3.1. Molecular Docking:** Therapeutically active chemicals of the plant such as such as Quercetin, Apigenin, Kaempferol and Malvidin were examined for binding affinity within the binding pocket of receptor protein having PDB ID code: 2GDE, 4MS3, 3CTT, 2CDU & 4Z69 for activities such as Anti-diabetic, Anthelmintic,

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Anticoagulant, Antioxidant and Anti-arthritic activities respectively using software PyRx. The binding score of PyRx is provided in Table 1.

Table 1. Docking scores for binding affinity of the active constituents

| **Chemical compounds** | **Docking score for Binding Affinity (Kcal/Mol)** | | | | |
|---|---|---|---|---|---|
| | **4Z69** | **2CDU** | **3CTT** | **4MS3** | **2GDE** |
| Quercetin | -9.9 | -8.8 | -8.5 | -7.8 | -8.0 |
| Apigenin | -10.8 | -8.2 | -9.7 | -7.1 | -8.2 |
| kaempferol | -9.8 | -8.9 | -8.5 | -7.8 | -8.2 |
| Malvidin | -9.8 | -7.2 | -7.9 | -6.9 | -7.3 |

**3.3. Antioxidant activity:** Antioxidant activity revealed that the fraction of ethanol extract had favourable radical scavenging potential with 88% inhibition (500 µg/ml) and had $IC_{50}$ value of 131.91±9.23 µg/ml while the reference marketed drug Ascorbic acid showed a inhibition of 93% at the same concentration with $IC_{50}$ value of 65.85±5.14µg/ml. The results at different concentrations are tabulated below in table 4.

Table 4: Percentage inhibition of *M. sylvestris* for *in-vitro* antioxidant activity

| **Extract solution** | **Percentage Inhibition at different concentrations** | | | |
|---|---|---|---|---|
| | **100µg/ml** | **300µg/ml** | **500µg/ml** | **$IC_{50}$ value(µg/ml)** |
| Ethanolic extract of *M. sylvestris* | 46 | 68 | 88 | 131.91±9.23 |
| Hexane extract of *M. sylvestris* | 33 | 49 | 61 | 333.32±11.13 |
| Chloroform extract of *M. sylvestris* | 35 | 53 | 63 | 295.24±10.15 |
| Ascorbic acid | 52 | 77 | 93 | 65.85±5.14 |

**3.4. Anti-arthritic activity:** The ethanolic fraction of *Malva sylvestris* at 500 µg/ml concentration had displayed the highest anti-denaturation of egg albumin with $IC_{50}$ value of 59.33±10.26 µg/ml when compared with Diclofenac which had $IC_{50}$ value of 47.78±16.20µg/ml at the same concentration. The results at different concentrations are tabulated below in table 5.

Table 5: Percentage inhibition of *M. sylvestris* for *in-vitro* antiarthritic activity

| **Extract solution** | **Percentage Inhibition at different concentrations** | | | |
|---|---|---|---|---|
| | **100µg/ml** | **300µg/ml** | **500µg/ml** | **$IC_{50}$ value(µg/ml)** |
| Ethanolic extract of *M. sylvestris* | 54 | 71 | 90 | 59.33±10.26 |
| Hexane extract of *M. sylvestris* | 53 | 69 | 88 | 71.42±11.20 |
| Chloroform extract of *M. sylvestris* | 45 | 58 | 73 | 176.18±9.20 |
| Diclofenac | 55 | 73 | 92 | 47.78±16.20 |

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**3.5. Antidiabetic activity:** In the α-amylase inhibition method for anti-diabetic activity, it was found that the ethanolic solution of different concentrations of *M. sylvestris* had greater inhibition potential of α-amylase with a maximum inhibition of 89% (at 500 µg/ml) with a $IC_{50}$ value 154.41±11.16 µg/ml. The standard drug Acarbose manifested 90% inhibition with $IC_{50}$ value of 101.68±12.16 µg/ml. This activity provides an insight that *M. sylvestris* can serve as a potential source for treatment of postprandial hyperglycaemia. The results at different concentrations are tabulated below in table 6.

Table 6: Percentage inhibition of *M. sylvestris* for *in-vitro* antidiabetic activity

| **Extract solution** | **Percentage Inhibition at different concentrations** | | | |
|---|---|---|---|---|
| | **100µg/ml** | **300µg/ml** | **500µg/ml** | **$IC_{50}$ value(µg/ml)** |
| Ethanolic extract of *M. sylvestris* | 47 | 63 | 89 | 154.41±11.16 |
| Hexane extract of *M. sylvestris* | 37 | 61 | 82 | 211.11±11.29 |
| Chloroform extract of *M. sylvestris* | 35 | 48 | 63 | 319.14±14.11 |
| Acarbose | 51 | 67 | 90 | 101.68±12.16 |

**3.6. Anticoagulant activity:** Using the Prothrombin method's coagulation time principles, an *in-vitro* anticoagulant activity test was conducted to determine *M. Sylvestris's* effectiveness as an anticoagulant agent in the blood samples of healthy normal individuals. All *M. sylvestris* extract solutions suppressed platelet aggregation and the most effective anticoagulant agent was the ethanolic extract which took 49.22 ±1.45 mins (at 500 mg/ml) to coagulate the extract concentration whereas the standard drug Warfarin had displayed a coagulation time of 50.12 ±1.19 mins. The ethanolic extract of *M. sylvestris* has the potential to function as an anticoagulant and a source of platelet inhibitors. The results at different concentrations are tabulated below in table 7.

Table 7: Prothrombin time of *M. sylvestris* for anticoagulant activity

| **Extract solution** | **Prothrombin time measurement (Mean ± SD) (min)** | | |
|---|---|---|---|
| | **100mg/ml** | **300mg/ml** | **500mg/ml** |
| Ethanolic extract of *M. sylvestris* | 19.12 ±1.31 | 31.11 ±1.17 | 49.22 ±1.45 |
| Hexane extract of *M. sylvestris* | 11.15 ±1.03 | 25.33 ±1.06 | 36.56 ±0.55 |
| Chloroform extract of *M. sylvestris* | 16.11 ±1.31 | 28.06 ±1.13 | 43.05 ±0.76 |
| Warfarin | 18.61 ±1.30 | 30.22 ±1.29 | 50.12 ±1.19 |

**3.6. Anthelmintic activity:** Among the extracts, ethanolic extract showed the most favourable result when compared with the reference Albendazole. Upon pouring the extract over the earthworms the motility was decreased which had caused paralysis followed by death and fading away of skin colours. Out of all the extract solutions that showed notable results, the ethanolic fraction had proved to be the most effective agent with a death time of 11 ± 0.53 minutes (100 mg/ml) and it may help prevent and treat gastrointestinal parasite infections in both humans and ruminants. The results at different concentrations are tabulated below in table 8.

**Table 8:** Paralysis and death time taken by the extract solution of *M. sylvestris* for anthelmintic activity

| **Extract solution** | **Concentration (mg/ml)** | **Time taken (in minutes)** | |
|---|---|---|---|
| | | **Paralysis** | **Death** |

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| | | | |
|---|---|---|---|
| Ethanolic extract of *M. sylvestris* | 25 | 27 ± 0.12 | 31 ± 0.16 |
| | 50 | 16 ± 0.53 | 24 ± 0.12 |
| | 100 | 6 ± 0.37 | 11 ± 0.53 |
| Hexane extract of *M. sylvestris* | 25 | 47 ± 0.17 | 51 ± 0.56 |
| | 50 | 36 ± 0.43 | 41 ± 0.32 |
| | 100 | 29 ± 0.27 | 35 ± 0.23 |
| Chloroform extract of *M. sylvestris* | 25 | 36 ± 0.27 | 39 ± 0.36 |
| | 50 | 24 ± 0.23 | 31 ± 0.42 |
| | 100 | 15 ± 0.29 | 19 ± 0.33 |
| Albendazole | 25 | 31 ± 0.52 | 36 ± 0.56 |
| | 50 | 20 ± 0.43 | 26 ± 0.52 |
| | 100 | 10 ± 0.56 | 15 ±0.55 |

# CONCLUSION

This research work focuses to shed light on the efficacy of *Malva Sylvestris* for medicinal applicability. The plant is highly valuable in ethnomedicine because it contains a variety of active constituents that enhance its therapeutic efficacy, making it important to domesticate and cultivate on a large scale. An attempt has been made in this current study to conduct *in vitro* studies of *Malva Sylvestris* ethanolic, hexane and chloroform solution for various activities such as antidiabetic, anticoagulant, anti-arthritic, antioxidant and anthelmintic activity. The plant's ethanolic extract was discovered to have the strongest effect on all of the *in vitro* activities. These biological activities are of great potential and significance for medicinal research so there is a need for further exploration to encourage the use of this medicinal plant as potential sources of new drugs.

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