RESEARCH PAPER

Phytochemical Investigation of Adenium obesum Plant Ethanolic Extract Fractions for Treatment of Rheumatoid Arthritis

Arshu Patel*[1], Trupti Kakade[1], Ajay Deshpande[2], Kishor Kothawade[3], Wasim Shaikh[4], Gajanan Daphal[4]

[1]Pravara Rural College of Pharmacy, Pravaranagar, Loni, Affiliated to Savitribai Phule Pune University, Pune, Maharashtra, India.
[2]Yashodeep College of Pharmacy, Saralgaon, Affiliated to Dr. Babasaheb Ambedkar Technological University, Lonere, Dist. Raigad, Maharashtra, India.
[3]Shree Gurudatta Shikshan Sanstha's College of Pharmacy, Manur, Affiliated to Savitribai Phule Pune University, Pune, Maharashtra, India.
[4]SSBT's Institute of Pharmacy, Bambhori, Jalgaon, Affiliated to Dr. Babasaheb Ambedkar Technological University, Lonere, Dist. Raigad, Maharashtra, India

Received: 14th Aug, 2025; Revised: 11th Sep 2025; Accepted: 18th Nov, 2025; Available Online: 30th Nov, 2025

ABSTRACT

Objectives: Adenium obesum has a long history of usage in management of inflammatory illnesses; the current research intends to learn more about its phytochemical composition and assess its anti-arthritic and anti-inflammatory efficacy in vitro using an ethanolic extract and column fractions.

Methods: The plant was gathered, identified and Soxhlet apparatus used to extract it ethanol. A preliminary phytochemical screening was conducted in order to find out important constituents. Seven solvent systems were used to carry out TLC profiling to assist in proper fractionation. Subsequently, column chromatography was done and four pooled fractions were produced onbase of comparable TLC pattern. The stability of HRBC membrane was used to assess anti-arthritic action in vitro, whereas suppression of COX-2 was used to test anti-inflammatory capability.The GC-MS based analytical method was used to conclude bioactive compound in crude extract.

Results:TLC profiling confirmed the presence of single spots with distinct Rf values across solvent systems. HRBC assay revealed that Fraction 4 showed the highest membrane stabilization at 100 µg/mL (91.80%), comparable to Aspirin (93.45%). In COX-2 inhibition assay, Fraction 3 exhibited the highest inhibition (72.08% at 100 µg/mL) associated to standard (85.06%). GC-MS analysis identified citronellal (64.37%), linalool (3.04%), and terpinen-4-ol (1.06%) among others, compounds well-documented for their anti-inflammatory activities.

Conclusion: Identification and extraction ethanol with Soxhlet apparatus was performed on the plant. The initial screen of phytochemical was carried out to determine significant constituents. TLC profiling was done in seven solvent systems that helped in proper fractionation. Column chromatography was then performed and four fractions were pooled according to shared similar pattern of TLC results. The in vitro determination of anti-arthritic action was performed via HRBC membrane stabilization test and inhibitory response was evaluated in a COX-2 test. Identification of the bioactive compound in crude extract was done based on the analytical method of GC-MS.

Keywords: *Adenium obesum, anti-arthritic, anti-inflammatory, HRBC assay, COX-2 inhibition, GC-MS, phytochemical analysis, column chromatography*

How to cite this article: Patel A, Kakade T, Deshpande A, Kothawade K, Shaikh W, Daphal G, Phytochemical Investigation of Adenium obesum Plant Ethanolic Extract Fractions for Treatment of Rheumatoid Arthritis. Int J Drug Deliv Technol. 2025;15(4): 1750-1761, DOI: 10.25258/ijddt.15.4.26

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic autoimmune illness that damagesexcellence of life of almost one percent of the entire world population, causes gradual destruction of joints and brings about functional disability and damage to health[1]. The World Health Organization reported that there are more than 20 million individuals worldwide with RA; the incidence of RA is increasing among women of the age bracket 30-60 years[2]. Its cost on the economy is enormous as the world spends over $46 billion every year on healthcare spending as a result of hospitalization, prolonged treatment and loss of productivity[3]. The existing medications such as NSAIDs, corticosteroids, and DMARDs only treat symptoms and they are commonly linked with strong side-effects that include ulcerative bowel disease, immune suppression, and toxicity to organs. In addition, low efficacy and being expensive have limited access to biologics particularly in the low- and middle-income countries[4]. Although new breakthroughs were made in the past few years regarding immunomodulatory treatments, the necessity to come up with safe, affordable, and effective substitutes is acute[5]. There has been curiosity in traditional medicinal plants as a conceivable therapeutic solution with bioactive phytochemicals having multi-

**Author for Correspondence: arshupatel59@gmail.com*

targeted action and reduced side effects, thus developing a promising tool in the treatment of RA[6].

*Adenium obesum*which is locally referred to as desert rose is a classical medicinal plant with abundant bioactive constituents, especially cardiac glycosides, flavonoids, terpenoids, and alkaloids[7]. Members of cardiac glycosides, as ouabain and oleandrin derivatives, are strong immunomodulators, as they can inhibit signaling pathways of the transcriptional factor NF-κB, leading to the inhibition of pro-inflammatory cytokine production (TNF-α, IL-1β, and IL-6)[8]. Flavonoids which in turn incorporate quercetin analogues have been detected to decrease oxidative stress and prevent infiltration of neutrophils in inflamed joints[9]. These compounds are lipophilic thus promoting the penetration of the membrane leading to cellular uptake in bioavailability. Moreover, its antioxidant and anti-inflammatory effect makes it a prospective disease-modifying anti-rheumatic agent. Although its pharmacological potential is high, its phytochemical depiction and fraction-dependent analysis of the medicinal value are unexplored[10].

In order to test the anti-arthritic action of plant-based composites, a number of in vitro models have been extensively used because they are reliable, reproducible and have relevance to the underlying mechanism[11]. HRBC membrane stabilization model is one of most utilized ones because it mimics stabilization of lysosomal membranes that can occur during inflammatory states, a key RA process. Likewise, the Cyclooxygenase-2 (COX-2) inhibition assay is a paramount framework of measuring the inhibitory characteristics of prostaglandin production which is the main inflammatory factor involved in destroying the joints and causing pains[12]. These models not only offer first chemicals to screen anti-inflammatory activity but also a means of ascertaining the therapeutic potential of natural products by modulating pathways of inflammation of interest. The fact that they are still applied in the initial stages of drug discovery affirms their relevance in the discovery of new drugs that can be used in management of chronic inflammatory conditions such as RA[13].

The current research paper tries to focus on phytochemical profile and medicinal value of ethanolic extract fractions of *Adenium obesum* in treating rheumatoid arthritis. Specific aims are fractionation and qualitative and quantitative phytochemical idealization as well as determination of anti-inflammatory outcome using in vitro and in vivo models. Study also aims at developing structure-activity relationships and adjusting a delivery method with increased bioefficacy.

MATERIALS AND METHOD

MATERIALS

Ethanol (analytical grade, 99.9%) was obtained from Research Lab Fine Chem Industries (Mumbai, India) and used for extraction. Silica gel (60–120 mesh, analytical grade) for column chromatography was procured from Neeta Chemicals (Pune, India). Sciquaint Chemicals of Pune, India, supplied the phosphate buffer saline (pH 7.4), electrolytes (potassium, sodium, and dihydrogen phosphate), and chloride ions. In order to conduct chromatographic profiling, Silica gel pre-coated TLC plates from Merck in Germany were used. Everything else that was employed was of an analytical grade chemical or reagent.

METHODS

Collection, Authentication and Preparation of plant material

The *Adenium obesum* species is a plant grown in the surroundings of the resident area of Loni, Taluka Rahata, District Ahmednagar, Maharashtra, India. In compliance with the standard protocols of collecting samples and with proper ethical standards, a voucher specimen of the plant collected was prepared and deposited with the Department of Botany, Padmashri Vikhe Patil College of Arts, Science & Commerce, Pravaranagar. The specimen was identified by Dr. A.S. Wabale and a reference number PVPC/bot./2024-25/304 allotted. This herbarium specimen was to be saved in the future information. Once the botanical identity is confirmed, the plant material is collected in large amounts and it is washed vigorously by running tap water, in order to remove dust, dirt and other surface contaminants. Clean plant parts were then chopped into small pieces and shade-dried over a period of 15 days in order to maintain phytochemical integrity. After drying the material was coarsely grounded with a mechanical grinder and maintained in an sealed container placed at room temperature until it is used in extraction and analysis[14,15].

Extraction of plant material using Soxhlet

Shade drying of the collected plant materials of *Adenium obesum* was carried out in a 15 day period and the dried samples were coarsely powdered and then extracted using solvents. The dried powder (100 g) was weighed, placed in a Soxhlet thimble and extracted in a Soxhlet extractor with 500 mL of ethanol, in a continuous extraction cycle of 8 hours. Ethanol was the solvent of choice in the extraction process because it perfectly dissolves a wide variety of phytoconstituents After the extraction was complete, the solution was filtered using Whatman No. 1 filter paper. The solvent was then allowed to evaporate at 24 °C under reduced pressure using a rotary evaporator. Prior to further phytochemical investigation and biological evaluations, the concentrated ethanolic extract was filtered out as a semi-solids mass, weighed to determine the yield, and stored in an airtight container at 4°C[16,17].

Phytochemical investigation of ethanolic extract

Ethanolic extraction of the *Adenium obesum* was subjected to a phytochemical screen initiated to identify the existence of important secondary metabolites considered to have therapeutic potential. Severe standard qualitative tests were performed on the extract to check the presence of different phytochemicals. The typical phytochemical protocols were followed to identify the groups of compounds by conventional chemical tests. These initial measurements gave a rudimentary idea of the bioactive components that were contained in the extract, which could also be matched to the observed pharmacological activity and be used as a starting point to more detailed research[18,19].

GC-MS Analysis of ethanolic extract

The objective was to start a GC-MS investigation to find the bioactive ingredients in the ethanolic extract of Adenium obesum. A GC-MS apparatus with a capillary column, helium as the carrier gas, and a maintained flow rate was used to perform the GC-MS analysis.The temperature in the oven was programmed to ramp up slowly between 50 °C and 280 °Cto enable the volatile compounds to separate. The injection carried 1 µL with splitless and temperature of injector and detector was retained at 250 °C and 280 °Crespectively. As a range, the mass spectra were recorded between 40–550 m/z. This was by comparing the obtained mass spectral fragmentation patterns and retention times with registered ones that were already available in the NIST library database of known compounds[20,21].

TLC profiling for selection of solvents for column chromatography

The ethanolic extract of *Adenium obesum* was subjected to TLC based on which appropriate solvent systems to carry out a successful column chromatography was established and also the TLC helped in determining some of the suitable solvents to be used in the column chromatography. Applying the extract to the previously coated silica gel TLC plate was done effectively using capillary tubes. The plates were then run in seven different solvent systems with different polarities: ethanol (100%), toluene:ethyl acetate (7:3), toluene:ethanol (5:5), ethyl acetate (100%), ethyl acetate:ethanol (5:5), chloroform:methanol (9:1), and chloroform: ethyl acetate (7:3). After letting the plates air dry, their fluorescence was examined using UV light with wavelengths of 254 and 366 nm. By slowly heating the plates that had been coated with the vanillin-sulfuric acid reagent, the extracted phytoconstituent bands were made visible. The number of spots, their Rf values, and the sharpness of the separation were recorded[22,23].

Column Chromatography

Ethanolic crude extract of *Adenium obesum* was fractionated using column chromatography based on TLC guided selection of solvents. Activated silica gel (60 120 mesh) provided in a clean glass column was wet packed in hexane to provide adequate standard settling of the stationary phase, and also to provide uniform packing. After 24h, the packed column was allowed to stay settled so as to stabilize the bed and to get rid of air. After stabilizing, the concentrated ethanolic extract was carefully placed to the top of the column after being pre-adsorbed on a little bit of silica gel and dried. Toluene:ethyl acetate (7:3), toluene:ethanol (5:5), ethyl acetate (100%), ethyl acetate:ethanol (5:5), chloroform:methanol (9:1), chloroform: ethyl acetate (7:3), and ethanol (100%) were the solvent systems used for the elution, which was carried out in increasing sequence of polarity.Each of the collected fractions were placed in labeled tubes and observed utilizing TLC in the progression of separation. Fractions with identical TLC patterns were combined, thickened under low pressure with a rotary evaporator and saved in sealed vials at 4 °C to be used again[24,25].

***In-vitro* anti-arthritic activity assessment of all collected fractions**

HRBC Suspension Method

The four collected fractions of the ethanolic *Adenium obesum* extract were tested for in vitro anti-arthritic actionby HRBC membrane stabilization assay. The fractions were generated by column chromatography. To get human blood, centrifuged tubes containing fresh blood were filled with heparin and spun at 3000 rpm for 10 minutes. After packing the cells into a tube and washing them twice with normal saline of the same volume, they were reconstituted as a 10% v/v suspension. To test the fractions, 1 mL of the test sample was mixed with 1 mL of the HRBC solution. The findings were then compared to concentrations of 20, 40, 60, 80, and 100 µg/mL. After 30 minutes of incubation at 56°C, the mixtures were centrifuged for 5 minutes at 2500 rpm. The absorbance of the supernatant was measured at 560 nm using a UV spectrophotometer.Between aspirin and normal saline, two drugs were used as standard reference and control, respectively.The anti-inflammatory effect was monitored by determining percent membrane stabilization[26,27].

COX-2 Inhibitory Activity

The four column chromatography fractions of *Adenium obesum* were tested in vitro in terms of inhibitory action against COX-2 via a fluorometric kit of COX-2 inhibitor screening assay, as outlined in the manufacturer protocol. Every test sample was prepared in ethanol and were tested at 20, 40, 60, 80 and 100 µg/mL. The standard reference inhibitor was the celecoxib (0.5 µM) and the blank was DMSO. The reaction mixtures were incubated at 25 C and fluorescence was monitored at excitation /emission wavelength set to 535/587 nm by a microplate reader over 10 minutes. The slope of each reaction was calculated by measuring relative fluorescence units (RFU) at two time points (T1 and T2) that overlapped in terms of the linear range. The percentage Activity of COX-2 was inhibited using the following formula:

$$\%\ Inhibition = \frac{Slope\ of\ enzyme\ control - Slope\ of\ sample}{Slope\ of\ enzyme\ control} \times 100$$

To ensure the accuracy and repeatability of the findings, every measurement was carried out three times[28,29].

RESULTS AND DISCUSSION

RESULTS

Preliminary phytochemical investigation

The ethanolic extract of *Adenium obesum* has revealedexistence of different bioactive constituents in its preliminary phytochemical screening as revealed by various bioactive constituents. Such categories of phytochemicals are renowned by their pharmacological relevance in anti-inflammatory, antioxidant, and immunomodulatory properties which justifies traditional use of plant. Fine outcomes of the screening are given in Table 1.

Table 1: Qualitative assessment of phytoconstituents in *Adenium obesum* ethanolic extract

Phytochemicals	**Test/reagent**	**Ethanolic extract of *Adenium obesum***
Alkaloids	Dragendorff Mayer Hager Wagner	- - - -
Glycosides	Keller-Killiani Borntrager's	+ +
Carbohydrates	Molisch Fehling	- -
Flavonoids	Shinoda Lead acetate	+ +
Steroids	Libermann-Burchard Salkowski	- -
Phenols	Ferric chloride	+
Saponins	Foam	-
Tannins	Lead acetate	+
Terpenoid	Copper Acetate	+

GC-MS of ethanolic extract

GC-MS analysis of ethanolic extract of *Adenium obesum* showed the existence of many bioactive phytoconstituents like terpenoids, monoterpenes, phenols, tannins and polyphenols. It should be noted that the most abundant compound was Citronellal (64.37%) followed by 1- 6-Undecadienic, Contact, Notification, Flag, Flag2, γ-Terpinene, (+)-2-Bornanone, Linalool, Caryophyllene, and Terpinen-4-ol, which are known to have the properties of anti-inflammatory and antioxidant. These results imply therapeutic prospective of extract to management of inflammation-provoked diseases. Chromatograms of each compound can be observed in Figure 1 and the comprehensive table of identified compounds are given in Table 2 with the retation times and relative peak areas

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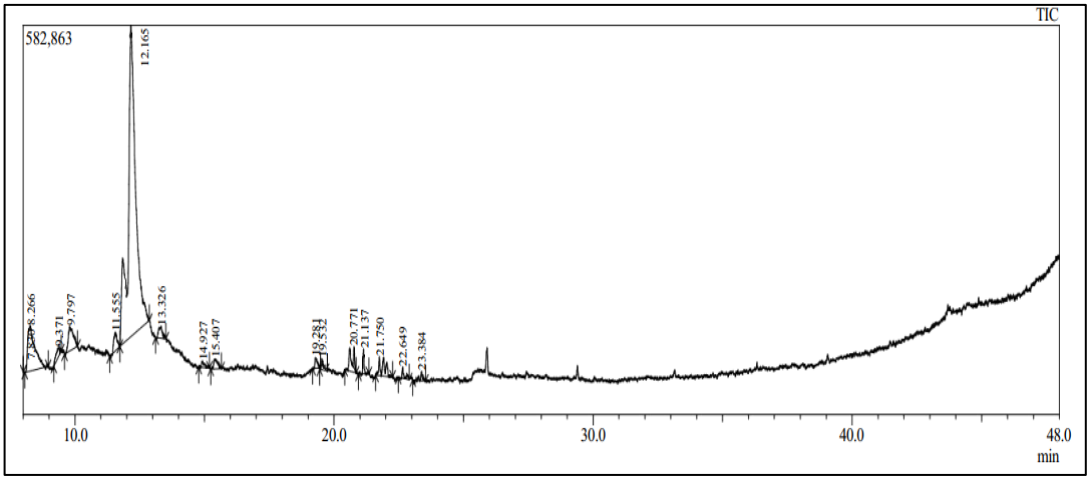

Figure 1: GC-MS spectra of *Adenium obesum* ethanolic extract

Table 2: GC-MS Analysis of Ethanolic Extract of *Adenium obesum*

Peak	R Time	Area %	Name	Identified Compounds
1	5.402	0.63	Propane, 1,1-diethoxy-2-methyl-	Phenol
2	6.576	11.42	gamma.-Terpinene	Terpenoid
3	7.543	0.55	Butane, 1,1-diethoxy-3-methyl-	Unknown
4	7.830	0.25	Ethane, 1,1,1-triethoxy-	Unknown
5	8.266	8.02	(+)-2-Bornanone	Tannin
6	9.371	0.85	(1R,2R,5S)-5-Methyl-2-(prop-1-en-2yl)cyclo	Polyphenol
7	9.797	3.04	Linalool	Monoterpene
8	11.55	1.41	(+)-2-Bornanone	Tannin
9	12.16	64.37	Citronellal	Monoterpene
10	13.32	1.06	Terpinen-4-ol	Volatile Phenol
11	14.92	0.50	Dodecane	Unknown
12	15.40	1.00	Benzene, 1-methoxy-4-methyl2-(1-methyleth	Phenol
14	19.28	0.57	alpha-Cubebene	Terpenoid
15	19.53	0.53	Copaene	Terpenoid
16	20.77	2.08	Caryophyllene	Terpenoid
17	21.13	0.73	cis-alpha-Bergamotene	Terpenoid
18	21.75	2.16	Humulene	Terpenoid
19	22.64	0.49	Naphthalene, decahydro-4amethyl-1-methyle	Unknown

TLC profiling of ethanolic extract

Seven solvent systems used in TLC profiling of the ethanol extract of *Adenium obesum* gave different spot separations that were useful in selection of mobile phases in column chromatography. System ethyl acetate:ethanol (5:5) gave one spot at R_f 0.62 (light brown) whereas toluene:ethyl acetate (7:3) showed a purple spot at R_f 0.68. Good resolution was seen in other systems such as ethyl acetate (R_f 0.52, yellow), ethanol (R_f 0.34, pale brown), and toluene:ethyl acetate:ethanol 6:2:2 (R_f 0.58, orange). Such findings were beneficial in ensuring optimum polarity to elute compounds. Table 3 presents codes of spot characteristics in detail

.

Table 3: TLC Profiling of Ethanolic Extract of *Adenium obesum*

Solvent System	Ratio (v/v)	R_f Value	Number of Spots	Spot Color (After Derivatization)
Toluene:Ethyl Acetate	7:3	0.62	3	Light brown, yellow
Toluene:Ethanol	5:5	0.55	2	Pale yellow, orange
Ethyl Acetate	100%	0.54	4	Yellow, pale orange
Ethyl Acetate:Ethanol	5:5	0.70	5	Brown, orange, yellow
Chloroform:Methanol	9:1	0.60	3	Light brown, reddish-brown
Chloroform:Ethyl Acetate	7:3	0.58	3	Orange, pale brown
Ethanol	100%	0.48	2	Faint yellow, blurred brown

TLC profiling of fractions collected after column chromatography

Fractionation performed after column chromatography of *Adenium obesum* ethanolic extract was successful as evident in the uniform separation patterns observed as a result of TLC profiling the respective fractions. Fraction 1 (F1) was eluted with toluene:ethyl acetate (7:3) toluene:ethanol (5:5) and gave a single spot with R_f values of 0.62 and 0.59 respectively both light brown. Fraction 2 (F2) that was made up in ethyl acetate (100%) formed a single yellow stain at R_f 0.54. Fraction 3 (F3) pooled of three solvent systems, ethyl acetate:ethanol (5:5), chloroform:methanol (9:1), and chloroform:ethyl acetate (7:3) gave consistent spots of light orange at R_f values of 0.63- 0.70. Fraction 4 (F4), recrystallized in ethanol (100%) gave a pale yellow spot at R_f 0.48. Such reproducible Rf values and spot properties confirm the need of pooling experiment and efficiency of the retrieved fractions, as presented in Table 4

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Table 4: TLC Profiling of Solvent Systems and Their Respective Pooled Fractions After Column Chromatography

Solvent System	Ratio (v/v)	R_f Value	Number of Spots	Spot Color	Pooled Fraction No.
Toluene:Ethyl Acetate	7:3	0.62	1	Light brown	Fraction 1 (F1)
Toluene:Ethanol	5:5	0.59	1	Light brown	Fraction 1 (F1)
Ethyl Acetate	100%	0.54	1	Yellow	Fraction 2 (F2)
Ethyl Acetate:Ethanol	5:5	0.70	1	Light orange	Fraction 3 (F3)
Chloroform:Methanol	9:1	0.68	1	Light orange	Fraction 3 (F3)
Chloroform:Ethyl Acetate	7:3	0.63	1	Light orange	Fraction 3 (F3)
Ethanol	100%	0.48	1	Pale yellow	Fraction 4 (F4)

HRBC assay

The anti-arthritic activity of the column fractions of *Adenium obesum* was investigated in vitro by the HRBC membrane stabilization test. Both fractions had concentration-dependent membrane stabilization activity; thus the ability to prevent hemolysis and inflammation may be involved. Fraction 4 recorded the highest protection of 91.80%, stabilized at 100 2 µg/mLthat is similar to standard aspirin (93.45%). This was subsequent to Fraction 3 (75.41%), Fraction 2 (71.31%), and Fraction 1 (64.75%) at the identical concentration. These findings imply that Fraction 4 has the strongest anti-arthritic effect of all the samples tested. The specific absorbance values and percentage stabilization have been recorded as given in Table 5, whereas, the corresponding graphical profile is depicted in Figure 2

.

Table 5: HRBC Assay for In Vitro Anti-Arthritic Action of Column Fractions of *Adenium obesum*

Sample	Concentrations (µl/ml)	Mean absorbance at 560 nm	Percentage of Stabilization
Control		1.22	-
Standard (Aspirin)	20	0.36	70.50%
	40	0.29	76.23%
	60	0.15	87.8%
	80	0.13	89.35%
	100	0.08	93.45%
Fraction 1	20	1.03	15.58%
	40	0.91	25.41%
	60	0.70	42.63%
	80	0.62	31.15%
	100	0.43	64.75%
Fraction 2	20	0.85	30.33%
	40	0.73	40.16%
	60	0.62	49.18%
	80	0.54	55.74%
	100	0.35	71.31%
Fraction 3	20	0.80	34.42%
	40	0.72	40.99%
	60	0.60	50.82%
	80	0.42	65.58%
	100	0.30	75.41%
Fraction 4	20	0.60	50.82%
	40	0.45	63.12%
	60	0.31	74.60%
	80	0.24	80.33%
	100	0.10	91.80%

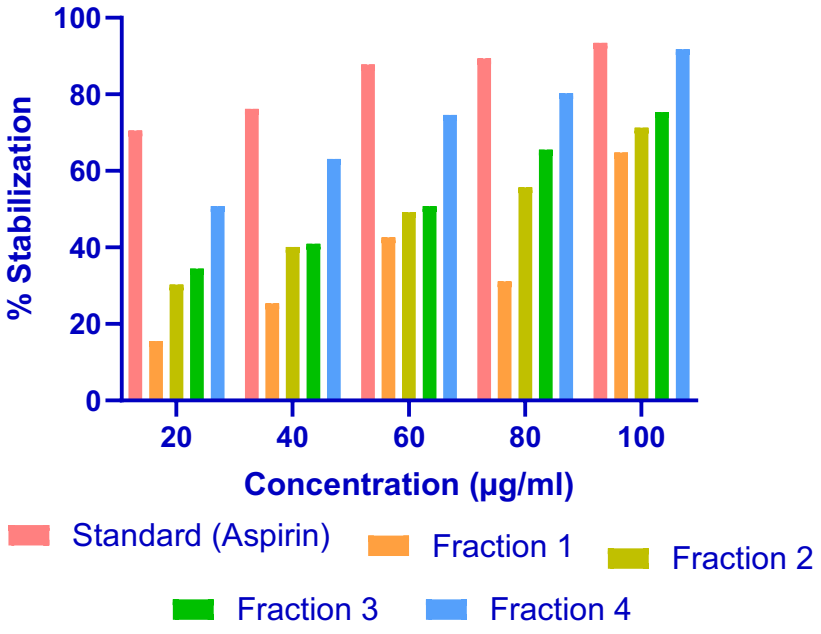

Figure 2: Percentage membrane stabilization of red blood cells by various fractions of *Adenium obesum* ethanolic extract compared to standard drug (Aspirin) at different concentrations (20–100 µg/mL).

COX-2 Inhibitory Activity

The fractions of *Adenium obesum* ethanolic extract were evaluated using the COX-2 inhibitory activity, which helps to find out their potential anti-inflammatory effects. Fraction 3 was the most inhibitory to 100.0 ug/mL (72.08%) closely followed by Fraction 4 (57.14%) and Fraction 2 (57.79%). Fraction 1 had a moderate effect and had an inhibition of 53.24%. Compared to a conventional drug aspirin that recorded an inhibition of 85.06 percent at the very concentration, the results are promising in terms of the COX-2 inhibition of the fractions, especially Fraction 3. Inhibition was seen to increase in concentration with all samples. The absolute values of absorbance and percent inhibition have been summarized on Table 6, comparative profile of inhibition has been displayed on Figure 3.

Table 6: COX-2 Inhibitory Activity of Column Fractions of *Adenium obesum* Ethanolic Extract Compared to Standard (Aspirin)

Sample	Concentration (µg/ml)	Mean Absorbance at 660nm	% Inhibition
Control		1.54	-
Standard(Aspirin)	20	1.32	14.28%
	40	0.91	40.90%
	60	0.76	50.64%
	80	0.52	66.23%
	100	0.23	85.06%
Fraction 1	20	1.49	03.24%
	40	1.22	20.77%
	60	1.06	31.16%
	80	0.82	46.75%
	100	0.72	53.24%
Fraction 2	20	1.52	1.30%
	40	1.46	5.19%
	60	1.25	18.83%
	80	0.92	40.26%
	100	0.65	57.79%
Fraction 3	20	1.31	14.94%
	40	1.17	24.03%
	60	0.86	44.16%
	80	0.62	59.74%
	100	0.43	72.08%
Fraction 4	20	1.42	7.79%
	40	1.18	23.38%
	60	1.05	31.82%
	80	0.92	40.26%

	100	0.66	57.14%

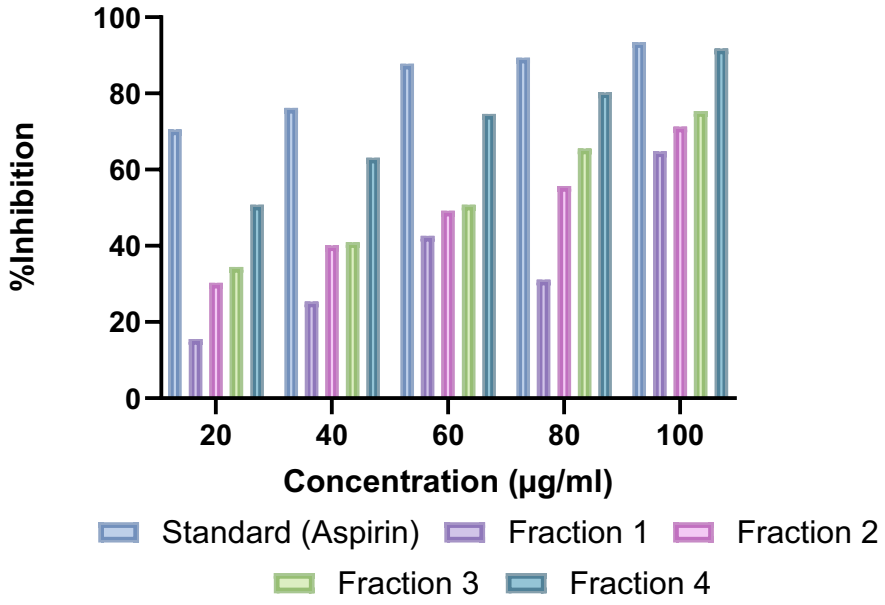

Figure 3: Percentage inhibition of COX-2 enzyme activity by different fractions of *Adenium obesum* ethanolic extract at various concentrations (20–100 µg/mL), compared to standard (Aspirin)

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DISCUSSION

The present research provides a significant set of evidence that the ethanolic extract of *Adenium obesum* has anti-arthritic and anti-inflammatory properties that were assessed in vitro and confirmed by a phytochemical and chromatographic analysis of the components[30]. Initial phytochemical screening of the crude ethanolic extract showed the isolated major secondary metabolites (Table 2)[31]. Such types of compounds are well coveted with potential to aid in the process of inflammation depending on their capacity to stabilize cell membranes, block cell membrane inductive enzymes (inflammatory mediators), and modulation of the inflammatory processes hence its inclusion as a potentially active component in the pharmacology[32]. TLC profiling of the ethanolic extract was carried out in seven systems of different polarity that resulted in nearly complete separation of ethanolic compounds and produced the extract in two runs[33]. The mode of separation revealed that a variability of different chemical constituents were present with the R_f value and the color of the spots proving the existence of different compounds (Table 3, Figure 2). The results formed the basis of column chromatography that was successfully used to obtain four pooled fractions based on similarity of TLC bands (Table 4). Clear and identical spots in multiple solvent systems guaranteed that individual fraction would reflect chemically different group of compounds[34].

The stability of the HRBC membranes was used in a stability assay that demonstrated the anti-arthritic complex potential by the capability of each fraction to stop the lysis of the red blood cells when exposed to hypotonic conditions. Fraction 4 was the most effective protective agent (91.80% stabilization at 100 µg/mL), which was the most effective to the same degree as standard aspirin (93.45%). Dose-dependent significant activity was also observed in fractions 3 and 2 which indicate the existence of the compounds that would preserve the integrity of the membrane under the influence of inflammatory conditions (Table 5, Figure 2). These results show the potential of the extract in inhibiting protein denaturation and cell lysis which are primary effects of an inflammatory form of arthritis. The COX-2 inhibition test was used to confirm further the anti-inflammatory activity. Fraction 3 was most potent with 72.08% inhibition at 100 µg/mLfollowed closely by Fraction 4 (57.14%). This dose-dependent inactivation of COX-2, an enzyme of core of the prostaglandin biosynthesis route, also supports the anti-inflammatory power of such fractions. Although aspirin had an excellent activity (85.06%), the prominent inhibitor displayed by the fractions suggests that the ethanolic extract contains constituents, which could influence enzymes involved in inflammation (Table 6, Figure 3)[35].

Notably, GC-MS sequencing of ethanolic crude extract exhibited the presence of 19 phytoconstituents, many of which have already been reported to be anti-inflammatory and analgesic (Table 1, Figure 1)[36]. In particular, citronellal, a common example of monoterpene, is the most predominant (64.37 peak area), and it has been tested to possess COX-2 inhibitory and anti-inflammatory characteristics[37]. Another detected compound is linalool that is said to have analgesic and membrane stabilizing effects[38]. In the same manner, terpinen-4-ol and (+)-2-bornanone have been revealed to have anti-arthritic roles

with the obstruct of inflammatory mediators and free radicals findings through earlier studies[39]. This discovery of these compounds gives a mechanistic explanation into the underlying biological behaviors and justifies the ethnomedicinal use of *Adenium obesum*.

CONCLUSION

Current study summarizes that ethanolic extract of *Adenium obesum* and chromatographically separated fractions, respectively, had enormous in vitro anti-arthritic and anti-inflammatory effects as supported by HRBC membrane stabilization tests and COX-2 inhibition studies. GC-MS assay revealed that various bioactive phytoconstituents like citronellal, linalool and terpinen-4-ol that have been shown to be effective in anti-inflammation areas are also contained in it. These results lend credence to the fact that *Adenium obesum* can be used as medicine and give rise to possible development of phytotherapeutic agents to treat rheumatoid arthritis. The premature formulation presents encouraging biological actions and can be used as a basis of further drug development. Additional in vivo pharmacological, toxicity and pre-clinical isolation of lead compounds will be required as part of establishing full clinical relevance and to provide assurances of safe therapeutic usage in the human population.

Abbreviations

HRBC: Human Red Blood Cell; COX-2: Cyclooxygenase-2; GC-MS: Gas Chromatography-Mass Spectrometry; TLC: Thin Layer Chromatography; Rf: Retention Factor; DMSO: Dimethyl Sulfoxide; PBS: Phosphate Buffered Saline; UV: Ultraviolet; HPLC: High Performance Liquid Chromatography; USP: United States Pharmacopeia.

Author's Contributions

All author contributed equally

Conflict of interest

Authors declare no conflict of interest regarding this study

Funding

Nil.

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REFERENCE

[1] Jahid M, Khan KU, Rehan-Ul-Haq, Ahmed RS. Overview of Rheumatoid Arthritis and Scientific Understanding of the Disease. Mediterr J Rheumatol 2023;34:284–91.

[2] Ashiq K, Ashiq S, Mobashar A, Abid F, Yasmeen A, Shehzadi N, et al. An updated review on rheumatoid arthritis (RA): Epidemiology, pathophysiology, diagnosis, and the current approaches for its treatment. Sudan J Med Sci 2023;18:539–51.

[3] Radu A-F, Bungau SG. Management of Rheumatoid Arthritis: An Overview. Cells 2021;10:2857. https://doi.org/10.3390/cells10112857.

[4] Dar E, Abbas M, Nazir S, Abbas M. Novel Management, Cure and Complications-Control of Rheumatoid Arthritis with Traditional and Modern Remedies (A Review). Pharm Chem J 2024;58:1020–8. https://doi.org/10.1007/s11094-024-03238-3.

[5] Khan S, Mohan K, Muzammil S, Alam MdA, Khayyam KU. Current Prospects in Rheumatoid Arthritis: Pathophysiology,Genetics, and Treatments. Recent Adv Anti-Infect Drug Discov 2024;19:36–55. https://doi.org/10.2174/2772434418666230406083149.

[6] Goodwin J. RHEUMATOID ARTHRITIS: Techniques for Massage Therapists: Take an in-depth look at adapting session planning for rheumatoid arthritis clients you see in your massage therapy practice. Massage Ther J 2023;62:26–56.

[7] Suleiman MHA, Brima EI. Phytochemicals, Trace Element Contents, and Antioxidant Activities of Bark of Taleh (Acacia seyal) and Desert Rose (Adenium obesum). Biol Trace Elem Res 2021;199:3135–46. https://doi.org/10.1007/s12011-020-02428-w.

[8] Kumavath R, Paul S, Pavithran H, Paul MK, Ghosh P, Barh D, et al. Emergence of Cardiac Glycosides as Potential Drugs: Current and Future Scope for Cancer Therapeutics. Biomolecules 2021;11:1275. https://doi.org/10.3390/biom11091275.

[9] Alharbi HOA, Alshebremi M, Babiker AY, Rahmani AH. The Role of Quercetin, a Flavonoid in the Management of Pathogenesis Through Regulation of Oxidative Stress, Inflammation, and Biological Activities. Biomolecules 2025;15:151. https://doi.org/10.3390/biom15010151.

[10] Dhankhar S, Kumar J, Chauhan S, Zahoor I, Wani SN, Saini M, et al. Flavonoids and flavonoid-based nanoparticles for the treatment of arthritis. Inflammopharmacology 2025;33:2193–216. https://doi.org/10.1007/s10787-025-01722-0.

[11] Bhoi A, Dwivedi SD, Singh D, Keshavkant S, Singh MR. Plant-Based Approaches for Rheumatoid Arthritis Regulation: Mechanistic Insights on Pathogenesis, Molecular Pathways, and Delivery Systems. Crit Rev Ther Drug Carr Syst 2024;41. https://doi.org/10.1615/CritRevTherDrugCarrierSyst.2023048324.

[12] Shah AA, Pandey N, Rai N, Bachheti RK, Gupta A. Exploring the Anti-inflammatory and Anti-arthritic Potential of Malus Peel Phenolics: A Comprehensive Comparative Analysis of 'Royal Delicious,' 'Delicious,' 'Golden Delicious,' and 'Maharaji' Cultivars from Kashmir, India. Appl Fruit Sci 2025;67:20. https://doi.org/10.1007/s10341-024-01240-3.

[13] Adek Zamrud Adnan, Fithriani Armin, Ikhwan Resmala Sudji, Mediola Dwi Novida, Dewi Imelda Roesma, Hana Afifah Ali, et al. IN VITRO ANTI-INFLAMMATORY ACTIVITY TEST OF TINOCRISPOSIDE AND FREEZE-DRIED AQUEOUS EXTRACT OF TINOSPORA CRISPA

STEMS ON HUMAN RED BLOOD CELL BY INCREASING MEMBRANE STABILITY EXPERIMENT. Asian J Pharm Clin Res 2019:125–9. https://doi.org/10.22159/ajpcr.2019.v12i5.30690.

[14] Baranauskaite J, Oçkun MA. Preparation and Characterization of Electrospun Polyvinylpyrrolidone Nanofiber Mat Containing Phillyrea Latifolia L. Herbal Extract and Evaluation of Antioxidant Mechanism. J Pharm Innov 2024;19:59. https://doi.org/10.1007/s12247-024-09867-1.

[15] Bhor RJ, Sable KS, Bhosale MS, Dighe SB. Synthesis and Anti Convulsant Activity of "N'-{4-[2-(1h-Benzimidazol-2-Yl)-2-Oxoethyl] Phenyl}-2-Hydroxyacetohydrazide and Its Derivatives." Adv Pharmacol Pharm 2023;11:46–56. https://doi.org/10.13189/app.2023.110105.

[16] Bontzolis CD, Dimitrellou D, Plioni I, Kandylis P, Soupioni M, Koutinas AA, et al. Effect of solvents on aniseed aerial plant extraction using soxhlet and ultrasound methods, regarding antimicrobial activity and total phenolic content. Food Chem Adv 2024;4:100609. https://doi.org/10.1016/j.focha.2024.100609.

[17] Mokaizh AAB, Nour AH, Alazaiza MYD, Mustafa SE, Omer MS, Nassani DE. Extraction and Characterization of Biological Phytoconstituents of Commiphora gileadensis Leaves Using Soxhlet Method. Processes 2024;12:1567. https://doi.org/10.3390/pr12081567.

[18] Ahmed N, Karobari MI, Yousaf A, Mohamed RN, Arshad S, Basheer SN, et al. The Antimicrobial Efficacy Against Selective Oral Microbes, Antioxidant Activity and Preliminary Phytochemical Screening of Zingiber officinale. Infect Drug Resist 2022;15:2773–85. https://doi.org/10.2147/IDR.S364175.

[19] Assiry AA, Ahmed N, Almuaddi A, Saif A, Alshahrani MA, Mohamed RN, et al. The antioxidant activity, preliminary phytochemical screening of Zingiber zerumbet and antimicrobial efficacy against selective endodontic bacteria. Food Sci Nutr 2023;11:4853–60. https://doi.org/10.1002/fsn3.3462.

[20] Ugwu OP-C, Alum EU, Okon MB, Aja PM, Obeagu EI, Onyeneke EC. Anti-nutritional and gas chromatography-mass spectrometry (GC-MS) analysis of ethanol root extract and fractions of Sphenocentrum jollyanum. RPS Pharm Pharmacol Rep 2023;2:rqad007. https://doi.org/10.1093/rpsppr/rqad007.

[21] Najda A, Bains A, Chawla P, Kumar A, Balant S, Walasek-Janusz M, et al. Assessment of Anti-Inflammatory and Antimicrobial Potential of Ethanolic Extract of Woodfordia fruticosa Flowers: GC-MS Analysis. Molecules 2021;26:7193. https://doi.org/10.3390/molecules26237193.

[22] Sharma A, Yu E, Morose G, Nguyen DT, Chen W-T. Designing Safer Solvents to Replace Methylene Chloride for Liquid Chromatography Applications Using Thin-Layer Chromatography as a Screening Tool. Separations 2021;8:172. https://doi.org/10.3390/separations8100172.

[23] Kowalska T, Sajewicz M. Thin-Layer Chromatography (TLC) in the Screening of Botanicals–Its Versatile Potential and Selected Applications. Molecules 2022;27:6607. https://doi.org/10.3390/molecules27196607.

[24] Babich OO, Samsuev IG, Tcibulnikova AV, Zemlyakova ES, Popov AD, Ivanova SA, et al. Properties of plant extracts and component composition: column chromatography and IR spectroscopy. Foods Raw Mater 2024;12:373–87.

[25] Grover M, Behl T, Sehgal A, Singh S, Sharma N, Virmani T, et al. In Vitro Phytochemical Screening, Cytotoxicity Studies of Curcuma longa Extracts with Isolation and Characterisation of Their Isolated Compounds. Molecules 2021;26:7509. https://doi.org/10.3390/molecules26247509.

[26] Pandey AK. Quantitative Estimation of Secondary Metabolite, In-vitro Antioxidant, Anti-Sickling & Anti-Inflammatory Activity by HRBC Membrane Stabilization of Ethanolic Extract of Bacopa monnieri (L.) Pennell. Adv Pharmacol Pharm 2024;12:19–33.

[27] Nakka H, Tiriveedhi A, Ande K, Kopuri S, Kandesi H. Phytochemical Analysis and In-vitro Anti-inflammatory Activity of Proboscidea louisianica Leaf Extract Using HRBC Membrane Stabilization Assay: Research Article. J Pharma Insights Res 2025;3:173–7. https://doi.org/10.69613/hrzgp133.

[28] Ahmadi M, Bekeschus S, Weltmann K-D, Woedtke T von, Wende K. Non-steroidal anti-inflammatory drugs: recent advances in the use of synthetic COX-2 inhibitors. RSC Med Chem 2022;13:471–96. https://doi.org/10.1039/D1MD00280E.

[29] Ragab MA, Eldehna WM, Nocentini A, Bonardi A, Okda HE, Elgendy B, et al. 4-(5-Amino-pyrazol-1-yl)benzenesulfonamide derivatives as novel multi-target anti-inflammatory agents endowed with inhibitory activity against COX-2, 5-LOX and carbonic anhydrase: Design, synthesis, and biological assessments. Eur J Med Chem 2023;250:115180. https://doi.org/10.1016/j.ejmech.2023.115180.

[30] Malik A, Najda A, Bains A, Nurzyńska-Wierdak R, Chawla P. Characterization of Citrusnobilis Peel Methanolic Extract for Antioxidant, Antimicrobial,

and Anti-Inflammatory Activity. Molecules 2021;26:4310. https://doi.org/10.3390/molecules26144310.

[31] Babafemi Tosin Ogunbiyi, Oluwaseyi Adegoke Adetunji, Olubunmi Esther Ogunbiyi, Gogonte Hezekiah Amah, Modupe Olusola Adetayo, Laide Omoyiola Olajide. In vitro antioxidant and anti-inflammatory activities of methanol extract of Vitellaria paradoxa seed (shea seed). World J Adv Res Rev 2021;11:057–65. https://doi.org/10.30574/wjarr.2021.11.3.0422.

[32] Somashekar G, Sudhakar U, Prakash SG, Suresh S, Srividya S, Rao SH. In-vitro Antioxidant and In-vitro Anti-inflammatory activities of Ethanolic leaves extract of Ormocarpum Cochinchinense. J Orofac Sci 2022;14:134. https://doi.org/10.4103/jofs.jofs_253_22.

[33] Samaraweera T, Samaraweera T, Senadeera NN, Ranaweera CB. In vitro Anti-Inflammatory Activity of Leaves of Jeffreycia zeylanica Using the Egg Albumin Denaturation Method and Human Red Blood Cell Stabilization Method. Asian Plant Res J 2023;11:56–64. https://doi.org/10.9734/aprj/2023/v11i6230.

[34] Chirumamilla P, Vankudoth S, Dharavath SB, Dasari R, Taduri S. In Vitro Anti-Inflammatory Activity of Green Synthesized Silver Nanoparticles and Leaf Methanolic Extract of Solanum khasianum Clarke. Proc Natl Acad Sci India Sect B Biol Sci 2022;92:301–7. https://doi.org/10.1007/s40011-021-01337-9.

[35] Saif Y. Khan* RDS. Evaluation of the anti-inflammatory and HRBC membrane stabilization potential of Kopsia fruticosa (Roxb.) A. DC 2025. https://doi.org/10.5281/ZENODO.15098915.

[36] Farooq S, Shaheen G, Asif HM, Aslam MR, Zahid R, Rajpoot SR, et al. Preliminary Phytochemical Analysis: In-Vitro Comparative Evaluation of Anti-arthritic and Anti-inflammatory Potential of Some Traditionally Used Medicinal Plants. Dose-Response 2022;20:15593258211069720. https://doi.org/10.1177/15593258211069720.

[37] Iqbal U, Malik A, Sial NT, Mehmood MH, Nawaz S, Papadakis M, et al. β-Citronellol: a potential anti-inflammatory and gastro-protective agent-mechanistic insights into its modulatory effects on COX-II, 5-LOX, eNOS, and ICAM-1 pathways through in vitro, in vivo, in silico, and network pharmacology studies. Inflammopharmacology 2024;32:3761–84. https://doi.org/10.1007/s10787-024-01569-x.

[38] Silva PR da, Pazos NDN, Andrade JC de, Sousa NF de, Pires HFO, Lima JL de F, et al. An In Silico Approach to Exploring the Antinociceptive Biological Activities of Linalool and its Metabolites. Mini Rev Med Chem 2024;24:1556–74. https://doi.org/10.2174/0113895575261945231122062659.

[39] Sun J, Sun P, Kang C, Zhang L, Guo L, Kou Y. Chemical composition and biological activities of essential oils from six lamiaceae folk medicinal plants. Front Plant Sci 2022;13. https://doi.org/10.3389/fpls.2022.919294.