DOI: https://doi.org/10.66220/vcx07w78 ISSN: 3139-9266

Journal of PhytoPharmaceutical and Advanced Chemistry (JPPAC)
https://jppac.in/index.php/jppac

Research Paper

Combined Effect of Leaf Extracts of *Mangifera indica* and *Plumeria alba* on Anti-Arthritic Activity

Harsh Saini, Arvind Kumar*, Mona Piplani, Pankaj Bhateja

Department of Pharmaceutical Chemistry, Maharaja Agrasen School of Pharmacy, Maharaja Agrasen University, Baddi, 174103, Himachal Pradesh, India.

*Corresponding author: arvindkumar@mau.edu.in

Article info:

Submitted: 19/08/2026
Revised: 25/09/2026
Accepted: 26/09/2026
Published: 05/10/2026
Manuscript ID: 00065
Subject: PhytoPharmaceutical Sciences

Keywords:

Mangifera indica, *Plumeria alba*, anti-arthritic activity, Total flavonoid content, Total phenolic content, Protein denaturation

Abstract:

This study was undertaken to investigate any combined antiarthritic effect between the methanolic extracts of leaves of *Mangifera indica* L. and *Plumeria alba* L. Both the extracts and their mixture (in the ratio 1:1) were studied for their total flavonoid content (TFC), total phenolic content (TPC) and antioxidant activity. *In-vitro* antiarthritic activity was also carried out using protein denaturation method. Both the individual extracts showed significant antioxidant activity but the mixture showed more activity compared to the individual extracts. The significant amount of phenol content in the extracts might be responsible for their antioxidant property. The results showed that the mixed extract exhibited the highest TPC (99.06 mg/g), surpassing *Mangifera indica* L. (81.40 mg/g) and *Plumeria alba* L. extract (75.25 mg/g). Similarly, the results of total flavonoid content revealed that the mixed extract exhibited the highest TFC (123.42 mg/g), as compared to *Mangifera indica* L. (98.42 mg/g) and *Plumeria alba* L. extract (78.22 mg/g). Both the individual extracts and their mixture showed a dose dependent inhibition of the protein denaturation. The values of IC_{50} for *Mangifera indica* L. extract and *Plumeria alba* L. extract were found to be 357.10 and 416.48 µg/mL, respectively. IC_{50} value of mixture was 246.36 µg/mL which was close to the IC_{50} value of standard drug diclofenac sodium (236.04 µg/mL). It can be concluded that a combination of *Mangifera indica* L. and *Plumeria alba* L. might exert an anti-arthritic effect due to a possibility of multifaceted biological activities shown by chemically different phytochemicals.

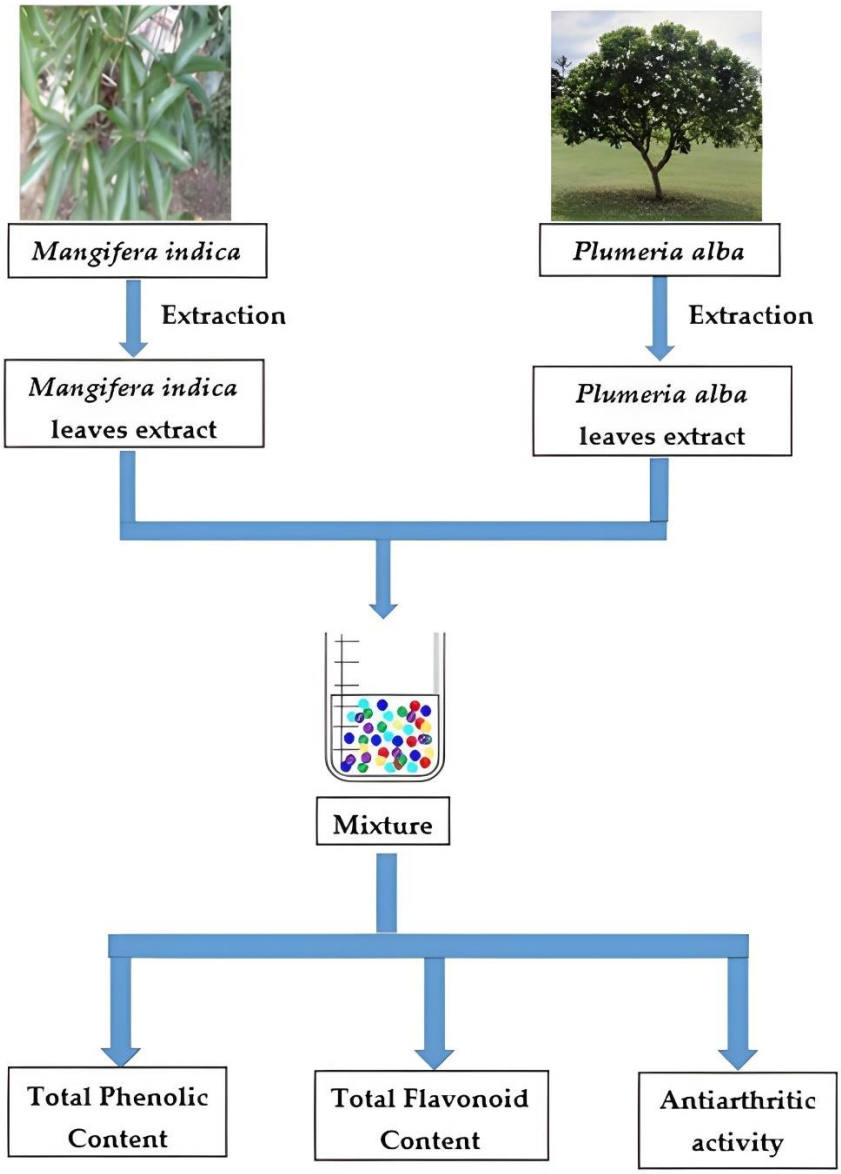
How to Cite this article: H. Saini, A. Kumar, M. Piplani, P. Bhateja. Combined Effect of Leaf Extracts of Mangifera indica and Plumeria alba on Anti-Arthritic Activity. *J. PhytoPharm. Adv. Chem.* **2026**, 01 [03], 190-211. DOI: https://doi.org/10.66220/vcx07w78

©2026 The authors

J. PhytoPharm. Adv. Chem. Volume: 01; Issue: 03; Year: 26; Pages: 190-211 H. Saini et al.
DOI: https://doi.org/10.66220/vcx07w78 ISSN: 3139-9266

This is an Open Access article distributed under the terms of the Creative Commons Attribution (CC BY NC), which permits unrestricted use, distribution, and reproduction in any medium, as long as the original authors and source are cited. No permission is required from the authors or the publishers. (https://creativecommons.org/licenses/by-nc/4.0/)

Graphical Abstract



1. Introduction

Arthritis is a common inflammatory disease and foremost cause of disability globally. It is an autoimmune disease characterized by pain, inflexibility and swelling [1]. Arthritis is of many different types but the major ones are osteoarthritis and rheumatoid arthritis. Most of the joint diseases affect synovial joints. One type of arthritis may have different symptoms than other type. Some have mild but some have severe symptoms. The common

DOI: https://doi.org/10.66220/vcx07w78 ISSN: 3139-9266

symptoms are: Pain, Swelling of Joints, Stiffness, Tenderness, Redness, Warmth, Stiffness, Bone Spurs, Limping, Difficulty in Standing and Walking, Fatigue (tiredness) [2-4]. Modern medications both the steroidal and non-steroidal anti-inflammatory drugs (NSAIDS) such as etoricoxib and disease modifying anti-rheumatic drugs such as methotrexate, hydroxychloroquine, sulphasalazine and leflunomide, are used for the symptomatic relief of the disease; but they provide only relieving effect and also cause some side effects [5]. This is the reason why patients with chronic musculoskeletal diseases use other methods to get relief from symptoms and use complementary and alternative medicine [6,7].

Oxidation, a physiological and chemical modification of biological systems and organic compounds, can be brought about by a number of physicochemical reactions, including light, heat or others oxidizing agents [8]. The oxidized bioactive molecules lead to the formation of reactive oxygen species (ROS) during aerobic metabolism or as a direct consequence of the exposure of extremely reactive molecules present in living tissues to the atmosphere [9]. ROS in the blood stream cause adverse effects to metabolic process due to their reaction with the free electron in the living system leading to development of many deadly diseases like ischemia, respiratory distress, arthritis, ageing, cancer and damaging many vital organs of the body. Herbal medicines possess a variety of phytoconstituents and are able to scavenge free radical oxygen species, which helps to reduce life-threatening diseases in our body [10]. Medicinal herbs have become very popular over the past several decades to replace the traditional way of improving the quality of life and maintaining health. Different plants' parts including roots, stems, leaves, barks and flowers have all been used medicinally [11]. Plant-based foods contain phytochemicals that have a variety of biological activities in addition to their antioxidant activity. Eating of foods rich in antioxidants has many health benefits that help in the prevention of diseases [12,13]. Most of the secondary metabolites like flavonoids, alkaloids, coumarins, and steroids have antioxidant and anticancer properties in both *in vivo* and *in vitro* models [14-18].

Mangifera Indica L. is a tropical plant found worldwide and is used both as a medicine and as an ornamental plant. It is used in the treatment of diarrhoea, dysentery, asthma, cough, bronchitis, anaemia, high blood pressure, insomnia, toothache, rheumatism, haemorrhage, leucorrhoea, and piles. It is also used as an astringent, antiseptic, dentifrice, diaphoretic, vermifuge, stomachic, laxative, tonic and diuretic. Mango is a widely consumed fruit and is hugely popular as traditional medicine in Southeast Asia and other subtropical and tropical countries [19,20]. It is considered as a good source of non-toxic antioxidants [21]. Besides the fruit, its leaves are also used in traditional medicine [22,23]. The leaves are rich in a range of bioactive constituents, including flavonoids, polyphenols, xanthones and terpenoids. Mangiferin is a prominent compound found in mango leaves, and has attracted considerable interest because of its diverse range of reported biological activities like anti-inflammatory, anti-microbial, anti-diabetic, hepato-protective and antioxidant activity [24-26]. The phytochemical profiles of leaves of *Mangifera indica* L. revealed that the major classes of compounds present are vitamins, minerals and phenolic compounds like phenolic acids, tannins, flavonoids, saponins, terpenoids and alkaloids [27]. Owing to their antioxidant, antimicrobial, anti-inflammatory and antidiabetic activities, they are considered as good candidates for treating several chronic diseases like neurodegenerative and cardiovascular diseases, diabetes and infections [28].

Plumeria alba L., commonly called white champa, is largely grown for its attractive and fragranced flowers. The seeds of *Plumeria alba* L. are used to stop bleeding. The milky sap from stem and leaves are used for skin diseases like scabies, herpes and ulcers [29]. Furthermore, its bark is battered and used as a plaster in firm tumours while the latter taxon is used as diuretic, purgative, cardiotonic, and hypotensive. *Plumeria alba* L. is rich in phytochemical composition, which is considered as a major contributor to its diverse pharmacological properties.

Various studies have been carried out on the isolation and identification of various bioactive chemicals, like flavonoids, terpenoids, saponins, iridoids, essential oils and phenolic acids. The therapeutic effects of these substances in both contemporary and traditional medicine have been attributed to their interaction [30]. The leaves

DOI: https://doi.org/10.66220/vcx07w78 ISSN: 3139-9266

are beneficial in bronchitis, rheumatism, inflammation, cholera, cough and cold and are also suitable as stimulant, antipyretic, antifungal and so forth [31]. Flavonoids, alkaloids and steroids are reported in the aerial parts [32]. The plant contains a mixture of iridoids, β sitosterol, amyrins, isoplumericin, plumeride coumerate, plumeride, plumeride coumerate glucoside and scopotein [33,34].

Both *Mangifera indica* L. and *Plumeria alba* L. are recognized for their ability to treat arthritis due to diverse types of active phytoconstituents. *Mangifera indica* L. and *Plumeria alba* L. together may have combined effects to treat arthritis. No literature is available on the study of combined effect of their mixtures. Therefore, the present study was undertaken to investigate any combined anti-arthritic effect between the methanolic extracts of leaves of *Mangifera indica* L. and *Plumeria alba* L.

2. Materials and Methods

2.1 Materials

Folin–Ciocalteu's phenol reagent, quercetin and gallic acid were obtained from Sigma-Aldrich (India). Methanol, sodium chloride, sodium carbonate, sodium hydroxide, potassium chloride, disodium hydrogen phosphate and potassium dihydrogen phosphate were obtained from Merck, India. All the above reagents were of analytical grade. Water was distilled out in the laboratory and used.

2.2 Sample Preparation

Mangifera indica L. and *Plumeria alba* L. leaves were collected from Hoshiarpur, Punjab. Plant materials were collected in the month of October 2025. Collected leaves (2 kg each) were washed under tap water to wash away the contaminations and air dried at 28-30°C. The samples were ground with a grinder and then screened through sieve of mesh size 40. The dried sample was stored in a cool and dry place until analysis to avoid chemical decomposition [11].

2.3 Extraction of *Mangifera indica* L. and *Plumeria alba* L.

The pulverized plant material was extracted by Soxhlet method by taking the powdered leaves at 60–65°C with 80% methanol. The percolate, after being filtered, was dried off by evaporating in a rotary evaporator. The dried crude extracts obtained were stored in airtight bottles at a temperature below 5°C. The percentage yield of the extracts obtained was 19% for *Mangifera indica* L. and 17% for *Plumeria alba* L [11].

2.4 Phytochemical Analysis

Chemical constituents were determined by qualitatively analyzing both the extracts. The standard procedure was carried out for the phytochemical screening of the extracts to confirm the presence of glycosides, carbohydrates, saponins, alkaloids, flavonoids, terpenoids and phenolic compounds [32].

2.5 Antioxidant Effect

The antioxidant effect of leaf extracts of *Mangifera indica* L. and *Plumeria alba* L. was tested using a 1,1-diphenyl-2-picryl hydrazyl (DPPH) technique [35].

2.5.1 DPPH Stock Solution (200 μg/mL):

30.0 mL methanol was added to 10 mg of DPPH taken in a 50.0 mL volumetric flask. The mixture was shaken until dissolved and volume was made up with methanol.

DOI: https://doi.org/10.66220/vcx07w78 ISSN: 3139-9266

2.5.2 Determination of DPPH λ_{max}:

2.5 mL of DPPH stock solution was taken into a 25.0 mL volumetric flask and the volume was made up with methanol to obtain a solution of 20 µg/mL DPPH concentration. The solution was scanned in the range 400-800 nm using UV spectrophotometer. The maximum wavelength (λ_{max}) was found to be 522 nm. Subsequently, the absorbance of this solution was studied every minute at the maximum absorbance and the time until absorbance reaches a stable value was recorded (operating time).

2.5.3 Test of Antioxidant Activity:

Antioxidant activity was tested based on the method reported by Nerdy and Manurung [35].

Method for Negative Control: 25 mL volumetric flask was taken and 5.0 mL of DPPH stock solution was added to it. Methanol was added to it to make the volume up to the mark to get a concentration of 40 µg/mL. Absorbance of the solution was taken at λ_{max} after leaving it till the operating time [35].

Method for Positive Control: 100.0 mL volumetric flask was taken and 100 mg of ascorbic acid was added to it along with 60.0 mL methanol. The mixture was shaken until dissolved and the volume was made up to the mark with methanol to get the solution with concentration 1000 µg/mL. Five 25.0 mL volumetric flasks were taken and 5.0 mL of DPPH stock solution was added to these flasks. Ascorbic acid stock solution 0.5 mL, 1.0 mL, 1.5 mL, 2.0 mL, and 2.5 mL of was added in these flask and volume was made up with methanol up to the mark. The solutions obtained had ascorbic acid concentration from 5 µg/mL to 25 µg/mL and DPPH concentration 40 µg/mL. Absorbance of each solution was taken at λ_{max} after leaving till the operating time [35].

Procedure for Sample: 100.0 mL volumetric flask was taken and 100 mg of the extract was added to it along with 60.0 mL methanol, shaken and diluted with methanol up to the mark to get the solution with concentration 1000 µg/mL. 5.0 mL of DPPH stock solution was added in five separate 25.0 mL volumetric flasks and 0.5, 1.0, 1.5, 2.0 and 2.5 mL of extract stock solution was added in each flask and volume was made up with methanol giving solutions with extract concentration 20 to 100 µg/mL and DPPH concentration 40 µg/mL. Absorbance of these solutions was measured at λ_{max} i.e. 522 nm [35].

Procedure for mixture of extracts: 100.0 mL volumetric flask was taken and 100 mg of the each of *Mangifera indica* L. and *Plumeria alba* L. extracts (1:1) were added to it along with 60.0 mL methanol, shaken and diluted with methanol up to the mark to get the solution with concentration 1000 µg/mL of each extract. 5.0 mL of DPPH stock solution was added in five separate 25.0 mL volumetric flasks and 0.5, 1.0, 1.5, 2.0 and 2.5 mL of extract stock solution was added in each flask and volume was made up with methanol giving solutions with extract concentration 20 to 100 µg/mL and DPPH concentration 40 µg/mL. Absorbance of these solutions was measured at λ_{max} i.e. 522 nm [35].

The free radical scavenging activity was calculated by the following equation:

$$\text{DPPH Scavenging Effect (\%)} = \frac{\text{Abs.}_{\text{negative control}} - \text{Abs.}_{\text{sample}}}{\text{Abs.}_{\text{negative control}}} \times 100$$

The result of free radical scavenging activity (%) was plotted on y-axis with sample concentration (µg/mL) on x-axis and Scavenging Concentration 50% (SC_{50} value) was calculated which shows the concentration of compound scavenging 50% free radical. Each sample was studied in triplicate.

DOI: https://doi.org/10.66220/vcx07w78 ISSN: 3139-9266

2.6 Total Phenolic Content (TPC)

Folin Ciocalteu (FC) reagent method was used to estimate the total amount of phenol in the extracts. In this method blue complex compounds are formed by the reaction of Folin Ciocalteu reagent with polyphenols that are present in the plant extract which are then quantified using UV-visible spectrophotometry. This blue colored complex is absorbed by the alkaline solution [36]. The total phenolic content is expressed in mg/g equivalent of gallic acid (GAE) while taking Gallic acid as a standard.

2.6.1 Preparation of Gallic Acid Stock Solution

A 10.0 mL volumetric flask was taken and 10 mg of gallic acid was added to it along with 6.0 mL methanol, shaken until dissolved and diluted with methanol to the mark to get 1000 μg/mL gallic acid stock solution.

2.6.2 Procedure for Standard

Five separate 10.0 mL volumetric flasks were taken and gallic acid stock solutions in the range 0.1 to 0.5 mL were added to these flasks and methanol was added to them to make up the volume to get standard gallic acid solutions having concentrations 10 μg/mL to 50 μg/mL.

Five separate 10.0 mL volumetric flasks were taken and 1 mL of each of the above gallic acid solution was added to these flasks followed by 5 mL distilled water and 0.5 mL FC reagent. These solutions were kept for 5 minutes followed by addition of 1.5 mL of sodium carbonate (20%) and then distilled water was added to make up the volume. The flasks were kept undisturbed for 2 hours at room temperature after covering with parafilm resulting in the development of an intense blue color. After 2 hours, the absorbance of these solutions were studied at 754 nm against the blank in UV-visible spectrophotometer. This estimation was repeated three times. Methanol was used to prepare the blank. Concentration of gallic acid (μg/mL) was plotted on x axis against absorbance on y axis to form the calibration curve.

2.6.3 Procedure for Sample (Individual Extract)

A 10.0 mL volumetric flask was taken and 10 mg of the extract was added to it followed by methanol, the solution was shaken followed by dilution with methanol up to the mark to get 1000 μg/mL extract stock solution. This stock solution was further diluted to get a 50 μg/mL extract solution. Another 10.0 mL volumetric flask was taken and 1 mL of this extract solution was added to the flask followed by 5 mL distilled water and 0.5 mL FC reagent. This solution was kept for 5 minutes followed by addition of 1.5 mL of sodium carbonate (20%) and then distilled water was added to make up the volume. The flask was kept undisturbed for 2 hours at room temperature after covering with parafilm. After 2 hours, the absorbance of the solution was studied at 754 nm against the blank in UV-visible spectrophotometer. The phenolic concentration in the sample was found from the regression equation [37-39]. Each sample was studied in triplicate.

2.6.4 Procedure for Mixture of Extracts

Two 10.0 mL volumetric flasks were taken and 10 mg each of *Mangifera indica* L. and *Plumeria alba* L. extract (1:1) was added to these separate flasks followed by methanol, the solutions were shaken followed by dilution with methanol up to the mark to get 1000 μg/mL each extract stock solution. These stock solutions were further diluted to get a 50 μg/mL extract solutions. Another 10.0 mL volumetric flask was taken and 0.5 mL of each of the above extract solution was added to the flask followed by 5 mL distilled water and 0.5 mL FC reagent. This solution was kept for 5 minutes followed by addition of 1.5 mL of sodium carbonate (20%) and then distilled water was added to make up the volume. The flask was kept undisturbed for 2 hours at room temperature after covering with

DOI: https://doi.org/10.66220/vcx07w78 ISSN: 3139-9266

parafilm. After 2 hours, the absorbance of the solution was studied at 754 nm against the blank in UV-visible spectrophotometer. The phenolic concentration in the sample was found from the regression equation [37-39]. Each sample was studied in triplicate.

The following equation was used to calculate TPC [40]:

$$\text{Total Phenolic Content} = \frac{C \cdot V \cdot DF}{W}$$

Where:

C is concentration (mg/mL) obtained from the standard plot
V is volume of sample (extract) (mL)
DF is Dilution Factor of sample solution
W is weight of dry extract (g)

2.7 Total Flavonoids Content (TFC)

TFC was measured with aluminium chloride colorimetric method [38,39,41,42].

2.7.1 Procedure for Preparing Quercetin Stock Solution

A 10.0 mL volumetric flask was taken and 100 mg of quercetin was added to it along with 6.0 mL methanol, shaken until dissolved and diluted with methanol to the mark to get 10 mg/mL quercetin stock solution.

2.7.2 Procedure for Standard

Five separate 10.0 mL volumetric flasks were taken and quercetin stock solutions in the range 0.2 to 1.0 mL were added to these flasks and methanol was added to them to make up the volume to get standard quercetin solutions having concentrations 200 μg/mL to 1000 μg/mL.

Five separate 10.0 mL volumetric flasks were taken and 1 mL of each of the above quercetin solution was added to these flasks followed by 4 mL distilled water and 0.3 mL of sodium nitrite solution (5%). These solutions were kept for 5 minutes followed by addition of 0.3 mL of aluminum chloride (10%). 2 mL of sodium hydroxide (1 M) was added at the 6th minute and then distilled water was added to make up the volume. Development of Orange yellowish color took place. The absorbance of these solutions were studied at 510 nm against the blank in UV-visible spectrophotometer. This estimation was repeated three times. Methanol was used to prepare the blank. Concentration of quercetin (μg/mL) was plotted on x axis against absorbance on y axis to get the calibration curve.

2.7.3 Procedure for Sample (Individual Extracts)

100.0 mL volumetric flask was taken and 50 mg of individual extract was added to the flask along with 60.0 mL methanol, shaken until dissolved and diluted with methanol to the mark to get each extract stock solution with concentration 500 μg/mL. A separate 10.0 mL volumetric flask were taken and 1 mL of extract solution was added to the flask followed by 4.0 mL distilled water and 0.3 mL of sodium nitrite solution (5%). The solution was kept for 5 minutes followed by addition of 0.3 mL of aluminum chloride (10%). 2 mL of sodium hydroxide (1 M) was added at the 6th minute and then distilled water was added to make up the volume. The absorbance was studied at 510 nm against the blank in UV-visible spectrophotometer. The total flavonoid concentration in the sample was

DOI: https://doi.org/10.66220/vcx07w78 ISSN: 3139-9266

found from the regression equation. Each sample was studied in triplicate. The total flavonoids content is expressed in mg/g equivalent of quercetin.

2.7.4 Procedure for Mixture of Extracts

Two 100.0 mL volumetric flasks were taken and 50 mg each of *Mangifera indica* L. and *Plumeria alba* L. extract (1:1) was added to these separate flasks followed by methanol, the solutions were shaken followed by dilution with methanol up to the mark to get 500 µg/mL each extract stock solution. Another 10.0 mL volumetric flask was taken and 0.5 mL of each of the above extract solution was added to the flask followed by 4.0 mL distilled water and 0.3 mL of 5 % sodium nitrite. The solution was kept for 5 minutes followed by addition of 0.3 mL of aluminum chloride (10%). 2 mL of sodium hydroxide (1 M) was added at the 6th minute and then distilled water was added to make up the volume. The absorbance was studied at 510 nm against the blank in UV-visible spectrophotometer. The total flavonoid concentration in the sample was found from the regression equation. Each sample was studied in triplicate.

The following equation was used to calculate TFC [40]:

$$\text{Total Flavonoid Content} = \frac{C \cdot V \cdot DF}{W}$$

Where:

C is concentration (mg/mL) obtained from the standard plot
V is volume of sample solution (extract) (mL)
DF is Dilution Factor of sample solution
W is weight of dry extract (g)

2.8 Anti-Arthritic Activity

2.8.1 Protein Denaturation Inhibition (Egg Albumin)

There is ample evidence that inflammatory and arthritic disease is caused by denaturation of tissue protein [17,43]. In some arthritic diseases, autoantigen production may be the result of protein denaturation. The agents inhibiting protein denaturation would be beneficial in developing anti-arthritic drugs [44]. The proteins, according to some literatures, become antigenic because of protein denaturation and macroglobulin formation and this activates the immune reaction and biochemical alterations in connective tissue, thereby developing rheumatoid arthritis [45,46].

The following solutions were prepared to study *in vitro* anti-arthritic activity:

Phosphate Buffer Saline (pH 6.4):

Sodium chloride (8.0 g), potassium chloride (0.2 g), KH_2PO_4 (0.24 g) and Na_2HPO_4 (1.44 g) were dissolved in 800 mL distilled water, pH of this solution adjusted to 6.4 using 1 N HCl and distilled water was added to make up the volume.

Test Solution (Individual Extract):

Egg albumin (0.2 mL) and phosphate buffer saline (2.8 mL) were added to 2 mL of individual extract with concentrations in the range 100 to 500 µg/mL to prepare 5 mL of test solution. Each sample was studied in triplicate.

DOI: https://doi.org/10.66220/vcx07w78 ISSN: 3139-9266

Test Solution (Mixture of Extracts):

Both the individual extracts were prepared in the concentration range 100 to 500 µg/mL. 1 mL of both these extracts (1:1) were taken and egg albumin (0.2 mL) and phosphate buffer saline (2.8 mL) were added to this mixture to prepare 5 mL of test solution. Each sample was studied in triplicate.

Control Solution:

Egg albumin (0.2 mL) and phosphate buffer saline (2.8 mL) were added to 2 mL of distilled water to prepare 5 mL of control solution. The control represents 100% protein denaturation.

Standard Solutions:

Egg albumin (0.2 mL) and phosphate buffer saline (2.8 mL) were added to 2 mL of varying concentrations of diclofenac sodium in the range 100 to 500 µg/mL to prepare 5 mL of standard solutions.

The samples were kept undisturbed at 37°C for 20 min. and then heated for 5 minutes at 70°C for denaturation. Absorbance of the samples was measured at 660 nm after cooling by using pure blank. Diclofenac sodium was used as standard drug. The percentage protein denaturation inhibition was calculated as follows:

$$\%\text{Inhibition} = \frac{(\text{Abs.}_{\text{Control}} - \text{Abs.}_{\text{Sample}})}{\text{Abs.}_{\text{Control}}} \times 100$$

where Abs. stands for absorbance

Anti-arthritic activity was expressed in IC_{50} values.

2.9 Statistical Analysis

The values reported are Mean ± Standard Deviation (SD). One-way analysis of variance (ANOVA) was used for the statistical analysis. The p values less than 0.05 were considered to be significant.

3. Results and Discussion

3.1 Phytochemical Screening

Preliminary phytochemical investigation of methanolic extract of *Mangifera indica* L. leaves showed the presence of carbohydrates, glycosides, flavonoids, alkaloids, terpenoids and saponins and phenols/tannins and whereas phytochemical analysis of methanolic extract of *Plumeria alba* L. showed the presence of glycosides, flavonoids, alkaloids, terpenoids and phenols/tannins (Table 1).

Table 1. Phytochemical screening of methanolic extracts of *Mangifera indica* L. and *Plumeria alba* L.

S. No.	Test	*Mangifera indica* L.	*Plumeria alba* L.
1.	Carbohydrates	+	-
2.	Glycosides	+	+
3.	Flavonoids	+	+
4.	Alkaloids	+	+

DOI: https://doi.org/10.66220/vcx07w78 ISSN: 3139-9266

5.	Terpenoids	+	+
6.	Saponins	+	-
7.	Tannins and Phenol	+	+

3.2 Antioxidant Effect

The antioxidant activity includes the ability to intercept the reactive oxygen species, to prevent or reduce the amount of radical species, and to generate less aggressive chemical species which may cause damage to the tissues [47]. The application of antioxidants has been a lot of interest because of their ability to protect against the damaging effect produced by reactive oxygen species [48]. In DPPH assay, a purple-colored solution takes up electrons and turns into a discolored solution. Color change correlates with effectiveness and antioxidant concentration. The level of discoloration is related to the free radical scavenging activity [22,49]. In DPPH method, the antioxidant activity test starts with the measurement of λ_{max} using spectrophotometer. The λ_{max} of DPPH solution was found to be 522 nm. The assay is not specific to any definite class of antioxidants and thus it gives a general antioxidant capability of the extract. The DPPH (stable radical) reduction activity of the crude extract can be used to study the radical scavenging activity and can reduce any molecule which would give up a hydrogen or electron to DPPH. The antioxidant activity of ascorbic acid, *Plumeria alba* L. leaves extract, *Mangifera indica* L. leaves extract and mixture of both the extracts are shown in Table 2.

Results of the average free radical scavenging activity were plotted against various concentrations of ascorbic acid, *Mangifera indica* L. leaves extract, *Plumeria alba* L. leaves extract and mixture of both the extracts as seen in Figure 1 to Figure 4.

Table 2. Antioxidant activity of ascorbic acid, *Mangifera indica* L. extract, *Plumeria alba* L. extract and mixture of extract.

Sample	Concentration (µg/mL)	Average Absorbance	Average Scavenging Activity (%)	Standard Deviation
Blank	0.00	0.74356	0.00	-
Ascorbic Acid	5.00	0.61435	17.38	0.37
	10.00	0.51233	31.10	0.77
	15.00	0.38093	48.77	1.03
	20.00	0.28634	61.49	0.65
	25.00	0.15139	79.64	1.40
***Mangifera indica* L. Leaves Extract**	20.00	0.53912	27.49	0.29
	40.00	0.44011	40.81	0.96
	60.00	0.28092	62.22	1.08
	80.00	0.14440	80.58	1.67
	100.00	0.03011	95.95	1.85
***Plumeria alba* L. Leaves Extract**	20.00	0.52956	28.78	0.25
	40.00	0.41721	43.89	0.85
	60.00	0.24894	66.52	1.54
	80.00	0.10395	86.02	1.51

DOI: https://doi.org/10.66220/vcx07w78 ISSN: 3139-9266

	100.00	0.01167	98.43	2.01
Mixture of Leaves Extract	20.00	0.43327	41.73	0.97
	40.00	0.31720	57.34	1.07
	60.00	0.20418	72.54	1.54
	80.00	0.08090	89.12	0.70
	100.00	0.00424	99.43	1.12

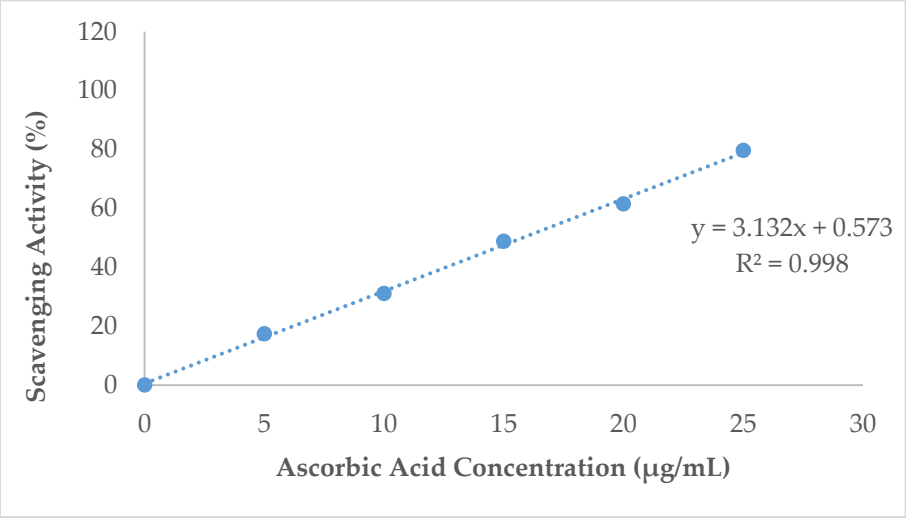


Figure 1. % Scavenging activity of ascorbic acid.

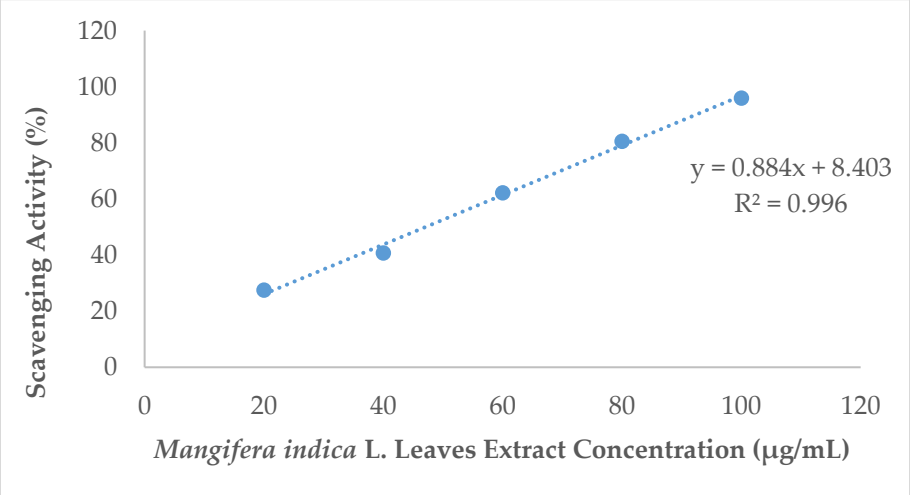


Figure 2. % Scavenging activity of *Mangifera indica* L. leaves extract.

DOI: https://doi.org/10.66220/vcx07w78 ISSN: 3139-9266

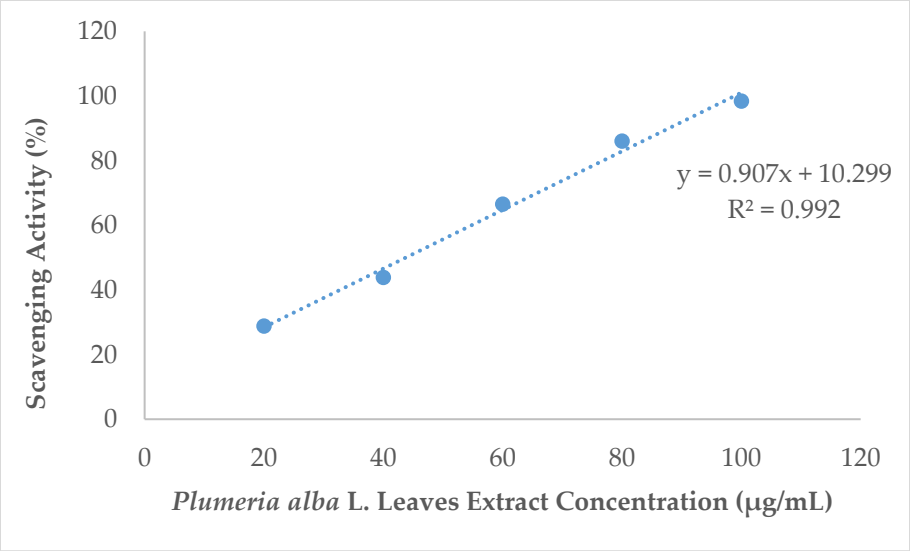


Figure 3. % Scavenging activity of *Plumeria alba* L. leaves extract.

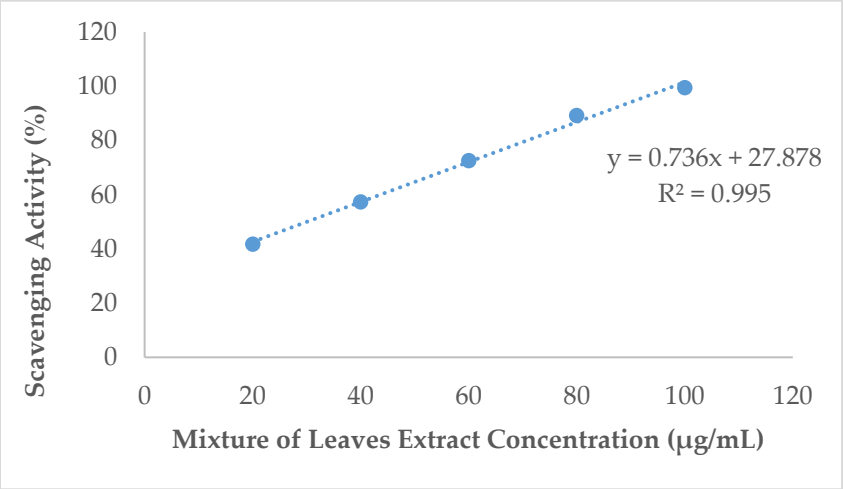


Figure 4. % Scavenging activity of mixture of *Mangifera indica* L. and *Plumeria alba* L. leaves extract.

A concentration-dependent change in radical scavenging activities was shown by all the crude extracts i.e., all the extracts showed an increase in the DPPH radical scavenging action with increase in their concentration. The correlation study between the concentration and scavenging activity for ascorbic acid, *Mangifera indica* L. extract, *Plumeria alba* L. extract and leaves extract of mixture gave a good correlation coefficient.

The values of SC_{50} concentration (the concentration which scavenges 50% of free radicals) were estimated using regression equations. The values of SC_{50} obtained were 15.78, 47.08, 43.76 and 30.06 µg/mL for ascorbic acid, *Mangifera indica* L. extract, *Plumeria alba* L. extract and leaves extract of mixture, respectively. The antioxidant activity of a compound increases with a decrease in the value of SC_{50} [50]. Literature suggests that highly active antioxidant activity was shown with SC_{50} value < 50 µg/mL whereas SC_{50} in the range 51-100 µg/mL are considered active, SC_{50} in the range 101-250 µg/mL moderately active, SC_{50} in the range 251-500 µg/mL less active, and anything above 500 µg/mL are considered inactive [35,51]. The results indicate that all of the leaves extracts have highly active antioxidant activity. The antioxidant activity of the *Plumeria alba* L. was higher than that of *Mangifera indica* L. extract, this could be due to higher phenolics content in *Plumeria alba* L. extract compared with *Mangifera indica* L. extract. This activity was more in the mixture of extracts of *Mangifera indica* L. and *Plumeria alba* L. than that of

DOI: https://doi.org/10.66220/vcx07w78 ISSN: 3139-9266

individual extract. The same enhanced effect was observed by Wilson *et al.* in polyherbal formulations containing Ficus species with respect to the antioxidant activity and antimicrobial activity [52].

Flavonoids, terpenes and phenols might be accountable for exhibiting the antioxidant activity in the leaves of *Mangifera indica* L. and *Plumeria alba* L. The phenolic compounds are potent antioxidants that may help to minimize the damage to the tissues caused by free radicals. The significant amount of phenolic content in the extracts might be accountable for their antioxidant property. The flavonoids act via the scavenging process or chelating process and they assist to increase the effect of antioxidants. The flavonoid composition in the extracts may also contribute to their antioxidant activity.

3.3 Total Phenolic Content (TPC)

Phenolic compounds are well established to play a significant role in antioxidants and are distributed in various plant species. Gallic acid was used as standard in Folin-Ciocalteu (FC) method to determine TPC. FC reagent reacted with reducing compounds such as polyphenols present in the extracts to form blue color which was spectrophotometrically measured at 754 nm. At this wavelength, maximum absorption was observed and was linearly dependent to the amount of phenolic content of the extracts. The absorbance data of gallic acid having different concentrations were plotted to make the standard curve (Figure 5).

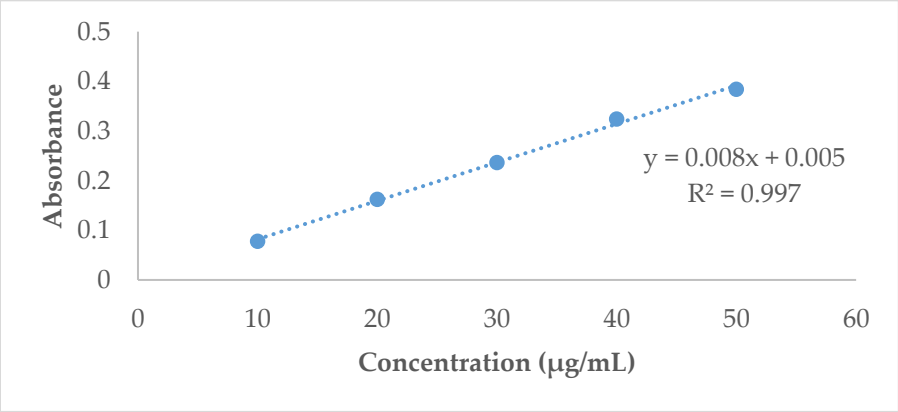


Figure 5. Standard plot of gallic acid for total phenolic content of different extracts.

Gallic acid standard curve was used to calculate gallic acid equivalent of different extracts and is given in Table 3. The results revealed that the mixed extract (1:1) exhibited the highest TPC (99.06 ± 2.04 mg/g), surpassing *Mangifera indica* L. extract (81.40 ± 1.65 mg/g) and *Plumeria alba* L. extract (75.25 ± 2.05 mg/g).

The antioxidant activity of the hydroalcoholic extract of *Plumeria alba* L. leaves was studied by Siang *et al.* and 71.04 mg GAE/g of TPC was reported [53]. Phuong *et al.* studied the antioxidant activity and TPC of *Mangifera indica* L. leaves alcoholic extract, and obtained the values of 76.39 mg GAE/g for TPC content [54].

Table 3. Total phenolic content of different extracts.

Extract	Concentration (in µg/mL)	Absorbance			Equivalent Gallic acid (mg/g ± SD)
		Sample No. 1	Sample No. 2	Sample No. 3	
***Mangifera Indica* L.**	50	0.314	0.327	0.313	81.40 ± 1.65
***Plumeria alba* L.**	50	0.293	0.305	0.284	75.25 ± 2.05
Mixture	50	0.377	0.392	0.389	99.06 ± 2.04

DOI: https://doi.org/10.66220/vcx07w78 ISSN: 3139-9266

3.4 Total Flavonoids Content (TFC)

Flavonoids, the water soluble polyphenolic compounds, are generally found in plants as glycoside. It is also well known that flavonoids are of great significance in human health and nutrition as they exhibit strong antioxidant activity shown by their scavenging and chelating action [53]. Hydroalcohol extracts were used to quantify the TFC by the aluminium chloride colorimetric assay with the aid of quercetin as a standard. The hydroxyl group at either the C-3 or C-5 position and keto groups at C-4 position of flavonols and flavones form acid stable complexes with aluminium chloride. In addition, it also forms liable complexes with ortho dihydroxide groups in A/B rings of flavonoids [38]. The absorbance data (Table 4) obtained at different concentration of quercetin were plotted against concentration to obtain a calibration curve (Figure 6). Results as shown in Table 4 demonstrated that the absorbance increased with increase in concentration of quercetin. The TFC of different extracts were determined by using the standard plot of quercetin and are given in Table 5.

Table 4. Total flavonoids standard estimation of the quercetin.

Sr. No.	Concentration (in µg/mL)	Average Absorbance	Standard Deviation
1.	200	0.142	2.12
2.	400	0.224	1.67
3.	600	0.365	0.34
4.	800	0.479	2.32
5.	1000	0.625	1.74

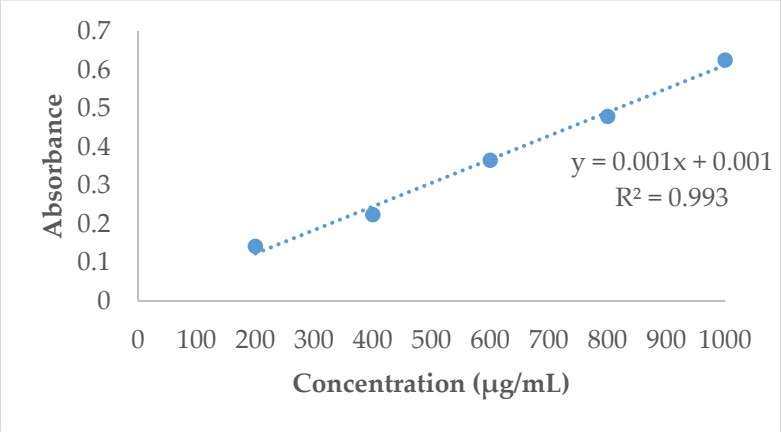


Figure 6. Standard plot of quercetin for total flavonoids content of different extracts.

The results revealed that the mixed extract (1:1) exhibited the highest TFC (123.42 ± 3.23 mg/g), surpassing *Mangifera indica* L. extract (98.42 ± 2.20 mg/g) and *Plumeria alba* L. extract (78.22 ± 2.14 mg/g) extracts.

Table 5. TFC of different extracts.

Extract	Concentration (in µg/mL)	Absorbance			Equivalent Quercetin (mg/g ± SD)
		Sample No. 1	Sample No. 2	Sample No. 3	
***Mangifera Indica* L.**	500	1.446	1.473	1.512	98.42 ± 2.20
***Plumeria alba* L.**	500	1.210	1.148	1.164	78.22 ± 2.14
Mixture	500	1.900	1.853	1.803	123.42 ± 3.23

DOI: https://doi.org/10.66220/vcx07w78 ISSN: 3139-9266

3.5 Anti-Arthritic Activity

Protein denaturation bioassay was used to assess the *in-vitro* anti-arthritic activity. One of the well-documented reason of tissue proteins denaturation causing inflammatory and arthritic diseases is the generation of autoantigens [55,56]. Therefore, in the development of anti-arthritic drugs, the agents which can prevent denaturation of protein would be worthwhile. The disruption of secondary structure, tertiary structure and quaternary structure of the molecules is involved in protein denaturation which finally results in cell death. It takes place due to stress conditions like a high temperature, high level of acidity and high level of salt. Denaturation is likely to be due to change in hydrogen, disulphide, hydrophobic and electrostatic bonding.

The percentage inhibition of protein denaturation was calculated for anti-arthritic activity and the values are given in Table 6–9 and shown in Figures 7-10.

Table 6. Effect of different concentrations of standard on protein denaturation.

Concentration (µg/mL)	% Inhibition			% Inhibition (Average)	Standard Deviation
	Sample No. 1	Sample No. 2	Sample No. 3		
100	23.61	24.23	24.95	24.26	0.67
200	44.17	41.16	42.21	42.51	1.53
300	67.05	65.63	66.48	66.39	0.71
400	79.74	75.31	80.27	78.44	2.72
500	94.43	93.67	97.74	95.28	2.16

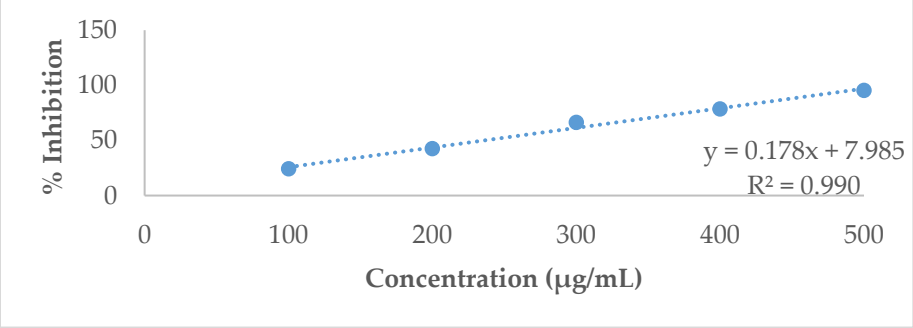


Figure 7. % Inhibition plot of standard.

Table 7. Effect of different concentrations of *Mangifera indica* L. leaves extract on protein denaturation.

Concentration (µg/mL)	% Inhibition			% Inhibition (Average)	Standard Deviation
	Sample No. 1	Sample No. 2	Sample No. 3		
100	16.95	16.34	15.89	16.39*	0.53
200	29.56	27.45	29.03	28.68*	1.10
300	41.71	42.79	41.01	41.84*	0.90
400	54.30	56.56	53.34	54.73*	1.65
500	69.45	71.03	69.95	70.14*	0.81

ANOVA was used to carry out statistical significance test between: Diclofenac sodium vs *Mangifera indica* L. extract.
*$p<0.05$.

DOI: https://doi.org/10.66220/vcx07w78 ISSN: 3139-9266

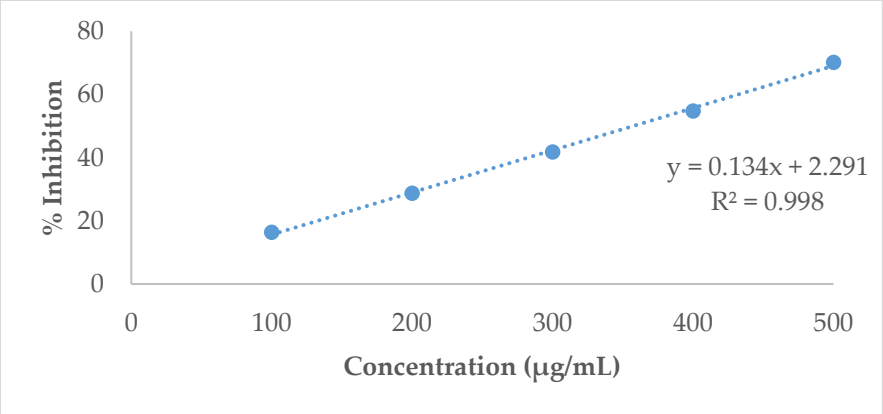


Figure 8. % Inhibition plot of *Mangifera indica* L.

Table 8. Effect of different concentrations of *Plumeria alba* L. leaves extract on protein denaturation.

Concentration (µg/mL)	% Inhibition			% Inhibition (Average)	Standard Deviation
	Sample No. 1	Sample No. 2	Sample No. 3		
100	15.32	15.02	14.56	14.97*	0.38
200	23.43	22.56	23.02	23.00*	0.44
300	32.12	31.31	32.69	32.04*	0.69
400	44.65	42.78	45.21	44.21*	1.27
500	65.43	63.84	65.91	65.06*	1.08

ANOVA was used to carry out statistical significance test between: Diclofenac sodium vs *Plumeria alba* L. extract.
*p<0.05.

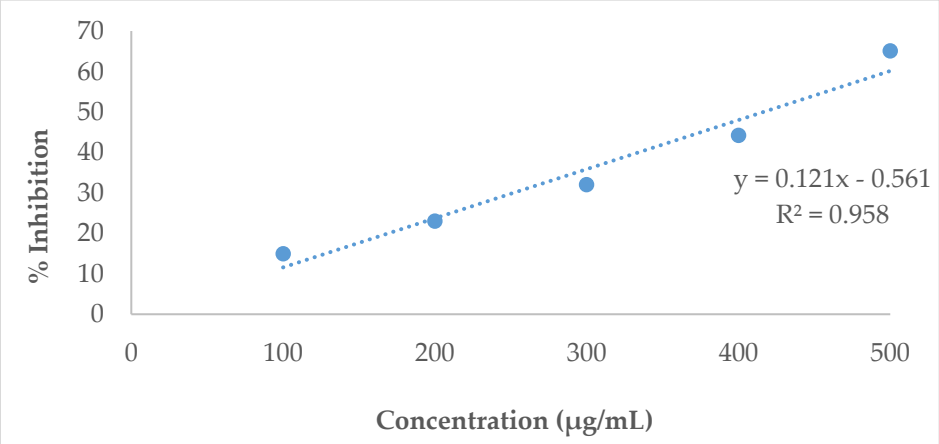


Figure 9. % Inhibition plot of *Plumeria alba* L.

Table 9. Effect of different concentrations of mixture of extracts on protein denaturation.

Concentration (µg/mL)	% Inhibition			% Inhibition (Average)	Standard Deviation
	Sample No. 1	Sample No. 2	Sample No. 3		
100	22.81	23.36	23.72	23.30^{NS}	0.46
200	40.82	38.35	40.78	39.98^{NS}	1.41
300	65.10	64.78	66.57	65.48^{NS}	0.95

400	75.63	76.34	72.45	74.81^{NS}	2.07
500	93.29	93.78	92.57	93.21^{NS}	0.61

ANOVA was used to carry out statistical significance test between: Diclofenac sodium vs Mixture.
NS: Non–Significant.

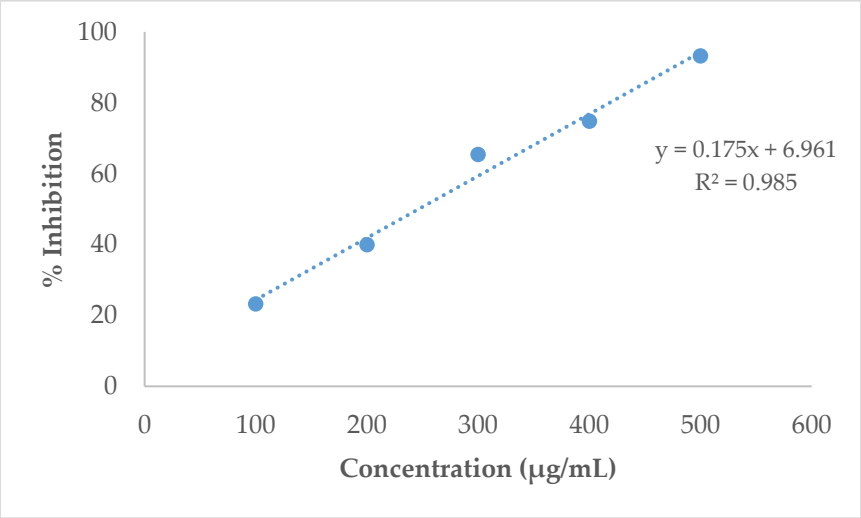


Figure 10. % Inhibition plot of mixture of extracts.

The extracts of *Mangifera indica* L. and *Plumeria alba* L. were determined to have IC_{50} value of 357.10 µg/mL and 416.48 µg/mL respectively (Table 10). Diclofenac sodium was used as a standard in this method for comparing the anti-arthritic activity and it was found that the mixture of extracts offered comparable anti-arthritic potential with an IC_{50} value of 246.36 µg/mL.

Table 10. IC_{50} of standard and different extracts in protein denaturation.

Group	IC_{50} (µg/mL)
Standard (Diclofenac sodium)	236.04
Mangifera indica L. extract	357.10
Plumeria alba L. extract	416.48
Mixture of extracts	246.36

There was a dose dependent protein denaturation inhibition shown by all the three extracts. However, when compared with their specific extracts, i.e. *Mangifera indica* L. extract and *Plumeria alba* L. extract, the mixture of extracts showed a higher percentage of protein denaturation inhibition. Similar effect was noted by Narayani *et al.* (2014) who investigated the enhanced effect of combined ethanolic extract of leaves of *Cardiospermum halicacabum* and *Calotropis gigantea* on *in-vivo* and *in-vitro* anti-arthritic activity. They found that bi-herbal extract showed a higher percentage of protein denaturation inhibition in comparison to their individual extracts but comparable with that of acetyl salicylic acid (the positive control) [57]. The presence of diverse chemical constituents in *Mangifera indica* L., *Plumeria alba* L. and their combination such as carbohydrate, phytosterol, phenol, tannins, glycosides, cardiac glycosides, flavonoids, lignin and terpenes might be accountable for the protein denaturation inhibition. It is generally believed that the overall useful medicinal value of different plants is due to a combined effect of different phytochemicals. These bioactive compounds could exert anti-rheumatic activity either by suppressing the synthesis of auto-antigens, or by preventing the denaturation of proteins, which is a characteristic feature of the rheumatic diseases [58].

DOI: https://doi.org/10.66220/vcx07w78 ISSN: 3139-9266

While both the plant extracts and their mixture demonstrated significant protection against protein denaturation, it is important to note that *in-vitro* protein denaturation inhibition is a preliminary screening method and cannot, by itself, demonstrate definitive antiarthritic efficacy. These results indicate a potential for anti-inflammatory activity by stabilizing proteins against thermal or chemical stress. However, they do not confirm that the extracts possess established therapeutic antiarthritic activity in a living system. Further evaluation in complex biological models is required to validate these findings.

4. Conclusion

Anti-arthritic effect of both *Mangifera indica* L. and *Plumeria alba* L. are reported but no literature is available on the study of combined effect of their mixtures. Hence, an attempt was made to explore any combined anti-arthritic action of the methanolic extracts of *Mangifera indica* L. and *Plumeria alba* L. leaves in the present study. The phytochemical analysis showed the presence of flavonoids, alkaloids, glycosides, terpenoids, phenol and tannins in both *Mangifera indica* L. and *Plumeria alba* L. leaves extracts. The antioxidant action of both the extracts and their mixture were investigated by using DPPH method. All these extracts displayed significant antioxidant action but the mixture showed more activity compared to the individual extracts. The significant amount of phenol content in the extracts might be responsible for their antioxidant property. These extracts were then studied for TPC and TFC. The results of TPC revealed that the mixed extract (1:1) exhibited the highest TPC surpassing *Mangifera indica* L. extract and *Plumeria alba* L. extract. Similarly, the results of TFC revealed that the mixed extract (1:1) exhibited the highest TFC in comparison to individual extract. These results further affirmed the results of antioxidant activity. Percentage inhibition was calculated using protein denaturation to carry out the i*n-vitro* anti-arthritic action of individual extracts and the mixture of extracts. Both the individual extracts and their mixture were able to inhibit the denaturation of protein in a dose-dependent mode. However, on comparison with the individual extracts, the mixture showed a higher percentage inhibition of protein denaturation. The percentage inhibition of the mixture was considerably comparable with diclofenac sodium at different concentrations. The IC_{50} values of the *Mangifera indica* L. extract and *Plumeria alba* L. extract were also calculated and found to be 357.10 and 416.48 µg/mL, respectively. IC_{50} value of the mixture (246.36 µg/mL) was comparable to the IC_{50} value of diclofenac sodium (236.04 µg/mL). The various chemical constituents in the plants, *Mangifera indica* L., *Plumeria alba* L. and their combination such as glycosides, phenols, alkaloids, flavonoids, terpenoids and tannins might be accountable for the protein denaturation inhibition. These bioactive constituents may either regulate the formation of auto antigens or protect against protein denaturation in the rheumatic diseases. In conclusion, a mixture of two herbal plants might exert a substantial anti-arthritic effect because of a possibility of discrete or multifaceted biological activities shown by chemically different phytochemicals. I*n vitro* assays usually fail to mimic the complex systemic physiology, pharmacokinetics, and multicellular interactions required to evaluate a plant extract's true anti-arthritic efficacy. Therefore, additional *in-vivo* and clinical studies are required to prove their activity and demonstrate their safety and usefulness medically. The primary limitation is that Further, it is also required to identify and isolate the active principles so as to explore the possible mechanism of protection against arthritis by this mixture of extracts.

Declarations

Conflict of Interest

The authors declare no conflict of interest.

Authors' Contribution

Harsh Saini: Literature survey, Experimental studies, Data acquisition, manuscript original draft preparation; Arvind Kumar: Concept, Design, Data analysis, Statistical analysis, manuscript review and editing, supervision; Mona Piplani: Concept, Design, Data analysis; Pankaj Bhateja: Data analysis

Funding

This research received no external funding

References

1. Vaijayanthimala P, Sakthipriya M, Sangameswaran B. *In vitro* anti-arthritic activity of *cissus quadrangularis* stem extract. Asian J Pharm Clin Res. 2019; 12(1): 250-252. doi: 10.22159/ajpcr.2019.v12i1.27353.
2. Harsh Mohan. Text book of pathology. Jaypee Brothers Medical Publishers (P) Ltd, Sixth Edition, 2010.
3. McInnes IB, Georg Schett. The pathogenesis of rheumatoid arthritis. N Engl J Med. 2011; 365(23): 2205-2219. doi: 10.1056/NEJMra1004965.
4. Dipiro JT. Pharmacotherapy: A pathophysiological approach. The McGraw-Hill Companies Inc, Seventh Edition, 2008.
5. Hazeena BV, Sadique J. Long term effect of herbal drug Withania somnifera on adjuvant induced arthritis in rats. Indian J Exp Biol. 1988; 26(11): 877–882.
6. Cronan TA, Kaplan RM, Posner L, Blumberg E, Kozin F. Prevalence of the use of unconventional remedies for arthritis in a metropolitan community. Arthritis Rheum. 1989; 32(12): 1604–1607. doi: 10.1002/anr.1780321217.
7. Singh S, Nair V, Gupta YK. Antiarthritic activity of Majoon suranjan (a polyherbal unani formulation) in rat. Indian J Med Res. 2011; 134(3): 384–388.
8. Brothersen C, McMahon DJ, Legako J, Martini S. Comparison of milk oxidation by exposure to LED and fluorescent light. J Dairy Sci. 2016; 99(4): 2537–2544. doi: 10.3168/jds.2015-9849.
9. Murphy MP. How mitochondria produce reactive oxygen species. Biochem J. 2009; 417(1): 1–13. doi: 10.1042/BJ20081386.
10. Khalid M, Alqarni MH, Foudah AI, Akhtar J, Shoaib A, Alam P. Evaluation of free radical scavenging potential of different bioactive fractions present in *Boerhavia diffusa* Linn. root extract: An *in-vitro* approach. J Pharm Res Int. 2020; 32(12): 99–107. doi: 10.9734/jpri/2020/v32i1230567.
11. Bhosle SD, Mahajan PS, Tilak PR, Deshmukh MA, Deodhar MN. Phytochemical screening, antibacterial and antioxidant activities of *Mangifera Indica* leaves. World Journal of Biology Pharmacy and Health Sciences. 2024; 19(1): 490–500. doi: 10.30574/wjbphs.2024.19.1.0451.
12. Nabavi SM, Ebrahimzadeh MA, Nabavi SF, Hamidinia A, Bekhradnia AR. Determination of antioxidant activity, phenol and flavonoid content of *Parrotia persica* Mey. Pharmacologyonline. 2008; 2: 560–567. doi: 10.1016/S2221-1691(12)60042-2.
13. Mishra KP, Ganju L, Sairam M, Banerjee PK, Sawhney RC. A review of high throughput technology for the screening of natural products. Biomed Pharmacother. 2008; 62(2): 94–98. doi: 10.1016/j.biopha.2007.06.012.
14. Mishima S, Matsumoto K, Futamura Y, et al. Antitumor effect of stilbenoids from *Vateria indica* against allografted sarcoma S-180 in animal model. J Exp Ther Oncol. 2003; 3(5): 283-288. doi: 10.1111/j.1533-869x.2003.01102.x.
15. Gupta M, Mazumber UK, Kumar RS, Kumar TS. Antitumor activity and antioxidant role of *Bauhinia racemose* against Ehrlich ascites carcinoma in Swiss albino mice. Acta Pharmacol Sin. 2004; 25(8): 1070–1076.
16. Gul MZ, Ahmad F, Kondapi AK, Qureshi IA, Ghazi IA. Antioxidant and antiproliferative activities of *Abrus precatorius* leaf extracts—an *in vitro* study. BMC Complement Altern Med. 2013; 13: article 53. doi: 10.1186/1472-6882-13-53.
17. Shilpa K, Chacko N, Shetty P, Savithri AS. Investigation of anti-arthritic activity (*in-vitro* models) of *Hibiscus hispidissimus* Griffith. The Journal of Phytopharmacology. 2018; 7(1): 60-65. doi: 10.31254/phyto.2018.7113.
18. Gul MZ, Attuluri V, Qureshi IA, Ghazi IA. Antioxidant and α-glucosidase inhibitory activities of *Murraya koenigii* leaf extracts. Pharmacognosy Journal. 2012; 4(32): 65–72. doi: 10.5530/pj.2012.32.12.
19. Galan Sauco V. Mango production and world market: current situation and future prospects. Acta Hortic. 2004; 645(645): 107–116. doi: 10.17660/ActaHortic.2004.645.7.

20. Hirano R, Oo TH, Watanabe KN. Myanmar mango landraces reveal genetic uniqueness over common cultivars from Florida, India, and Southeast Asia. Genome. 2010; 53(4): 321–330. doi: 10.1139/g10-005.
21. Nunez Selles AJ, Velez Castro HT, Aguero-Aguero J, et al. Isolation and quantitative analysis of phenolic antioxidants, free sugars, and polyols from mango (*Mangifera indica* L.) stem bark aqueous decoction used in Cuba as a nutritional supplement. J Agric Food Chem. 2002; 50(4): 762–766. doi: 10.1021/jf011064b.
22. Itoh K, Matsukawa T, Okamoto M, et al. *In vitro* antioxidant activity of *Mangifera indica* leaf extracts. J Plant Stud. 2020; 9(2): 39-45. doi: 10.5539/jps.v9n2p39.
23. Molla MM, Sabuz AA, Chowdhury MGF, et al. Nutritional composition, antioxidant activity and common phytochemicals of selected BARI mango varieties and commercial cultivar langra: phytochemical and antioxidant activities of BARI mango varieties. Eur J Agric Food Sci. 2020; 2(6): 1-8. doi: 10.24018/ejfood.2020.2.6.151.
24. Ediriweera MK, Tennekoon KH, Samarakoon SR. A review on ethnopharmacological applications, pharmacological activities, and bioactive compounds of *Mangifera indica* (mango). Evid Based Complement Alternat Med. 2017; 2017: 6949835. doi: 10.1155/2017/6949835.
25. Kumar M, Saurabh V, Tomar M, et al. Mango (*Mangifera indica* L.) leaves: nutritional composition, phytochemical profile, and health-promoting bioactivities. Antioxidants (Basel). 2021; 10(2): 299–323. doi: 10.3390/antiox10020299.
26. Samanta S, Chanda R, Ganguli S, Gopi Reddy A, Banerjee J. Anti-diabetic activity of mango (*Mangifera indica*): a review. MOJ Bioequivalence & Bioavailability. 2019; 6(2): 23-26. doi: 10.15406/mojbb.2019.06.00131.
27. Mehmood H, Mehmood J, Zulfiqar N. Exploring the phytochemistry and pharmacology of *Mangifera indica* L. (Mango) leaves: A review. Int J Plant Based Pharm. 2024; 4(4): 9-18. doi: 10.29228/ijpbp.38.
28. Ngo DH. Biological activities of mango leaves (*Mangifera indica*). Journal of Thu Dau Mot University. 2025; 7(2): 588-600. doi: 10.37550/tdmu.EJS/2025.02.657.
29. Raju RA. Wild Plants of Indian Sub-Continent and Their Economic Use. CBS Publishers and Distributors, First Edition, 2000.
30. Zaufeen M, Rahim SA. Medicinal potential of *Plumeria Alba* Linn: A review of its phytochemistry and pharmacological activities. Int J Sci Res Sci Technol. 2025; 12(3): 1273-1275. doi: 10.32628/IJSRST25123137.
31. Baghel AS, Mishra CK, Rani A, Sasmal D, Nema RK. Antibacterial activity of *Plumeria rubra* Linn. plant extract. J Chem Pharm Res. 2010; 2(6): 435-440.
32. Evans WC. Trease and Evans, Pharmacognosy. Saunders Elsevier, Fifteenth Edition, 2002.
33. Rengaswami S, Venkatarao E. Chemical components of plumeria alba. Proceedings of the Indian Academy of Science. 1960; 52: 173–181.
34. Coppen JJW, Cobb AL. The occurrence of iridoids in Plumeria and Allamanda. Phytochemistry. 1983; 22(1): 125–128. doi: 10.1016/S0031-9422(00)80071-0.
35. Nerdy N, Manurung K. Spectrophotometric method for antioxidant activity test and total phenolic determination of red dragon fruit leaves and white dragon fruit leaves. Rasayan J Chem. 2018; 11(3): 1183-1192. doi: 10.31788/RJC.2018.1134018.
36. Folin O, Ciocalteu V. On tyrosine and tryptophane determinations in proteins. J biol Chem. 1927; 73(2): 627-650. doi: 10.1016/S0021-9258(18)84277-6.
37. Bhalodia N, Nariya P, Acharya R, Shukla V. Evaluation of *in vitro* antioxidant activity of flowers of *Cassia fistula* Linn. Int J PharmTech Res. 2011; 3(1): 589-599.
38. Kamtekar S, Keer V, Patil V. Estimation of phenolic content, flavonoid content, antioxidant and alpha amylase inhibitory activity of marketed polyherbal formulation. Journal of Applied Pharmaceutical Science. 2014; 4(9): 61-65. doi: 10.7324/JAPS.2014.40911.
39. Patel A, Patel A, Patel A, Patel NM. Estimation of flavonoid, polyphenolic content and *in vitro* antioxidant capacity of leaves of *Tephrosia purpurea* Linn. (Leguminosae). Int J Pharma Sci Res. 2010; 1(1): 66-77.

40. Sumaiyah, Masfria, Dalimunthe A. Determination of total phenolic content, total flavonoid content, and antimutagenic activity of ethanol extract nanoparticles of R*haphidophora pinnata* schott leaves. Rasayan J Chem. 2018; 11(2): 505-510. doi: 10.31788/RJC.2018.1122068.
41. Pallab K, Tapan B, Tapas P, Ramen K. Estimation of total flavonoids content (TFC) and antioxidant activities of methanolic whole plant extract of *Biophytum sensitivum* Linn. J Drug Delivery Ther. 2013; 3(4): 33-37. doi: 10.22270/jddt.v3i4.546.
42. Satish Kumar T, Baskar R, Shanmugam SK, Rajasekaren P, Sadasivam S, Manikandan V. Optimization of flavonoids extraction from the leaves of *Tabernaemontana Heyneana* Wall. Using L16 orthodesign. Nature and Science. 2008; 6(3): 10-21.
43. Alamgeer. Uttra, AM, Hasan UH. Anti-arthritic activity of aqueous-methanolic extract and various fractions of Berberis orthobotrys Bien ex Aitch. BMC Complement Altern Med. 2017; 17(1): 371. doi: 10.1186/s12906-017-1879-9.
44. Kumari CS, Yasmin N, Raffiq M, Hussain N, Babuselvam M. *In vitro* anti-inflammatory and anti-arthritic property of *Rhizopora mucronata* leaves. Int J Pharm Sci Res. 2016; 6(3): 482-485.
45. Lavanya R, Maheshwari SU, Harish G, Raj JB, Kamali S, Hemamalani D. Investigation of *in vitro* anti-inflammatory, anti-platelet and antiarthritic activities in the leaves of *Anisomeles malabarica* Linn. Res J Pharm Biol Chem Sci. 2010; 1(4): 745-752.
46. Srividya S, Sridevi G. Anti-arthritic and anti-inflammatory activity of ethanolic leaf extract of *Ormocarpum sennoides*. Int J Pharm Pharm Sci. 2016; 8(5): 117-121.
47. Kumar Y, Kumar VS. Comparative antioxidant capacity of plant leaves and herbs with their antioxidative potential in meat system under accelerated oxidation conditions. J Food Meas Charact. 2020; 14(1): 3250–3262. doi: 10.1007/s11694-020-00571-5.
48. Mohan CG, Deepak M, Viswanatha GL, et al. Anti-oxidant and anti-inflammatory activity of leaf extracts and fractions of *Mangifera indica*. Asian Pac J Trop Med. 2013; 6(4): 311–314. doi: 10.1016/S1995-7645(13)60062-0.
49. Kumar S, Sandhir R, Ojha S. Evaluation of antioxidant activity and total phenol in different varieties of *Lantana camara* leaves. BMC Res Notes. 2014; 7: 560. doi: 10.1186/1756-0500-7-560.
50. Wright RJ, Lee KS, Hyacinth HI, et al. An investigation of the antioxidant capacity in extracts from *Moringa oleifera* plants grown in Jamaica. Plants (Basel). 2017; 6(4): 48. doi: 10.3390/plants6040048.
51. Jun M, Fu HY, Hong J, Wan X, Yang CS, Ho CT. Comparison of antioxidant activities of isoflavones from Kudzu root (*Pueraria lobata* Ohwi). Journal of Food Science. 2003; 68(6): 2117-2122. doi: 10.1111/j.1365-2621.2003.tb07029.x.
52. Wilson V, Shetye SS, Kaur K, Shetty S. Study of synergistic effects on antioxidant activity and antimicrobial activity of polyherbal formulations containing Ficus species. Int J Pharm Pharm Sci. 2016; 8(4): 50-53.
53. Siang CC, Akmal Ali Baig M, Sarath Chandiran I, et al. Antioxidant and antibacterial response of hydroalcoholic extract of *Plumeria alba* leaves. International Journal of Health Sciences. 2022; 6(S2): 12393–12411. doi: 10.53730/ijhs.v6nS1.8703.
54. Phuong NTN, Ha MT, Nguyen DXT, et al. Development and antioxidant evaluation of mango leaf (*Mangifera indica* L.) extract loaded silk fibroin nanoparticles. Front Mater. 2024; 11: 1-12. doi: 10.3389/fmats.2024.1419697.
55. Vallabh deshpande, Varsha Jadav M, Kadam VJ. *In-vitro* anti arthritic activity of *Abutilam indicum* (Linn.) sweet. J Pharm Res. 2009; 2(4): 644645.
56. Umapathy E, Ndebia E, Meeme A, Adam B, Menziwa P, Chungag BN. An experimental evaluation of *Albuca setosa* aqueous extract on membrane stabilization, protein denaturation and white blood cell migration during acute inflammation. J Med Plant Res. 2010; 4(9): 789–795. doi: 10.5897/JMPR10.056.
57. Narayani PC, Anbu J, Vasuki R, Rajeswari H. *In vitro* and *in vivo* anti-arthritic activity of combined ethanolic extracts of *Calotropis gigantea* and *Cardiospermum halicacabum* in Wistar rats. Journal of Natural Remedies. 2014; 14(1): 58-66. doi: 10.18311/jnr/2014/90.

DOI: https://doi.org/10.66220/vcx07w78 ISSN: 3139-9266

58. Padmini N, Sundaramoorthy SD, Tripathi H, Hari R. *In vitro* and *in vivo* anti-arthritic activity of combined ethanolic extracts of *Pisonia grandis* and *Cardiospermum halicacabum* in Wistar rats. Journal of Applied Pharmaceutical Science. 2016; 6(9): 102-108. doi: 10.7324/JAPS.2016.60915.