https://doi.org/10.47552/ijam.v16i1.5317 ISSN No: 0976-5921

International Journal of Ayurvedic Medicine, Vol 16 (1), 2025; 52-63

Quantitative estimation of secondary metabolites, in-vitro antioxidant, anti-inflammatory and anti-sickling activity of leaf of *Ficus virens* Aiton.

Research Article

Abhishek Kumar Pandey[1*], Ajay Kumar Harit[2], Kamlesh Sonekar[3]

1. Assistant Professor, 3. Lab attendant, Department of Botany,
2. Associate Professor Department of Zoology,
Kalinga University, Naya Raipur, Chhattisgarh. India.

Abstract

Sickle cell anaemia is a severe global disease marked by oxidative stress and painful episodes. This study evaluates the antioxidant, anti-inflammatory, and anti-sickling properties of the ethanolic extract of *Ficus virens Aiton* leaves. Total phenolic (TPC), tannin (TTC), and flavonoid (TFC) contents were assessed spectrophotometrically, while antioxidant activity was measured using DPPH, anti-inflammatory activity via the HRBC method, and anti-sickling effects through Emmel and reversibility tests. The extract showed high TPC (79.84±2.77 µg/mg GAE), TTC (10.51±0.62 µg/mg GAE), and TFC (132.62±6.69 µg/mg QE). It significantly reduced haemolysis (12.19±1.84% at 2000 µg/ml) and exhibited strong antioxidant activity (89.34±0.56% DPPH scavenging at 75 µg/ml, IC50: 22.76±1.01 µg/ml). The extract also demonstrated a high sickle cell reversal rate (84.13±0.25% at 10 mg/ml after 150 min), comparable to phenylalanine (85.71%). These results highlight *Ficus virens Aiton* as a promising candidate for treating inflammation and sickle cell anaemia.

Keywords: *Ficus virens* Aiton, Anti-inflammatory, Anti-oxidant, Total phenolic content, Sickle cell anaemia.

Introduction

Sickle cell anaemia is a major concern throughout the globe. Thousands of children die due to its lethality every year around the world. As we know haemoglobin molecules undergo this change by replacing glutamic acid, a polar amino acid, with valine, a non-polar amino acid in position six (1,2,3). It is a result of the defective gene (mutation) on chromosome number 11. The mutant haemoglobin polymerises inside the red blood cells (RBCs) at low oxygen tension, resulting in a drastic reduction in the red cells deformability. Upon polymerisation and precipitation of sickle haemoglobin (HbS) within erythrocytes, sickle cells change from their normal spherical shape into one resembling a sickle. Single nucleotide substitution (Thymine in place of Adenine) allows HbS to polymerise when deoxygenated since valine can dock with complementary sites on adjacent globin chains (4). Herrick demonstrated sickle-shaped RBCs in human blood for the first time in 1910 (5, 6).

It has been seen in sickle cell anaemia, the body suffers the problem of inflammation and oxidative stress. The pathophysiology of sickle cell disease (SCD) is characterised by inflammation (7). A tissue-resident cell of the innate immune system detects damaging agents and activates nearby neutrophils. Activated monocytes migrate to the inflamed tissue, recruit inflammatory monocytes and promote proinflammatory conditions (8, 9). SCD is characterised by an increase in leukocyte count and activation of granulocytes, monocytes, and platelets (10, 7, 11). Different types of proinflammatory mediators derived from various sources including leukocytes, platelets, and endothelial cells such as tumour necrosis factor-alpha (TNF-α) and the interleukins (IL), IL-6, IL-1β, and IL-8, are recorded in higher amount in sickle cell patients (12, 13,14). The multiplied production and release of proinflammatory cytokines can promote the vaso-occlusive procedure because of endothelial activation, erythrocytes, and leukocyte adhesion to vascular endothelial and endothelial cell apoptosis (15).

In Chhattisgarh, Sickle cell anaemia is a major concern and is one of India's most affected state (16). Indeed, the prevailing neighbouring states such as Maharashtra, Odisha, Jharkhand, Madhya Pradesh, and parts of Andhra Pradesh are also suffering the state of India. In Chhattisgarh, sickle-shaped haemoglobin is common in the central and southern regions of states which are Raigarh, Jashpur, Surguja, and Koriya. Chhattisgarh tribe with a high prevalence percentage of sickle cell haemoglobin is as follows: Halba in Rajnandgaon and Durg districts, Muria and Hillmaria in the Bastar district. Studies also show that scheduled caste and some OBC groups such as Sahu, Chandrakar, Kurmi, and Yadav in the south and central west of Chhattisgarh also have sickle-shaped haemoglobin. It's about the same ratio of a tribe in this area. Additionally, there are also 12 districts in Chhattisgarh, which are

*** Corresponding Author:**
Abhishek Kumar Pandey
Assistant Professor, Department of Botany,
Kalinga University, Naya Raipur,
Chhattisgarh, India-492101
Email Id: abhishek.pandey@kalingauniversity.ac.in

classified as the main area carrying SCD which includes Dantewada, Bastar, Kanker, Korba, Mahasamund, Rajnandgaon, Dhamtari, Kawardha, Bilaspur, Durg, Raipur, and Janjgir-Champa (16).

Indeed, out of the total population of the Chhattisgarh state that is 7.5 million (16) Gond tribe (20% of the total tribal population) is strongly affected by sickle cell disease. Sickle cell disease screened 359,823 subjects among 2,087 (99.7%) of the villages in Raipur District, Chhattisgarh State, India between October 2007 and June 2010. The sickle cell trait (sickle cell gene comes from a single parent) occurred in 33,467 (9.30%) and individuals with sickle cell disease (sickle cell gene comes from both parents) 747 (0.21%) (16). A previous study conducted in the Rajnandgaon district, also documented that 9.75% of the examined population carries a sickle cell gene (17). In another study, random sampling of 6088 people was done to test the sickle cell anaemia problem by slide test method, and a total of 249 (4.09%), people were found sickled positive. Further electrophoresis test was performed for all 249 of which 67 were found homozygous (HbSS) and 182 were found heterozygous (HbAS) positive in Rajnandgaon district (18).

Many plants were investigated for anti-sickling agents. Researchers around the globe are working on different medicinal plants and checking the potential of many phytoconstituents against this disease. The present study also aims to investigate the therapeutic potential of *Ficus virens Aiton* against inflammation, oxidative stress, and sickle cell anaemia. *Ficus virens Aiton* belongs to the Moraceae family found in the Indian sub-continent commonly known as white fig in English. It is a sacred tree in Indian methodology and is believed to be the dwelling place of lord Vishnu. In Ayurveda, this plant is mentioned in the name of *Plaksha*. According to Ayurveda, this plant has been used in urogenital diseases. Ethnobotanical bark decoction is used to strengthen the bone, bark powder is used to prevent bleeding and in the treatment of wounds. Fruits are used to treat diarrhoea (19). Fresh and young leaves of the plant are edible in some parts of China (20) Recent studies suggest that plants contain anti-viral (21), and anti-cancerous activity (22). The main objective of this study is to explore the antioxidant, anti-inflammatory, and anti-sickling activity of this plant (*Ficus virens Aiton*) and provide some alternative and effective plant-based drugs for the treatment of the above-mentioned problem.

Material and Methods

Collection of plant material

The plant sample has been collected from the Raghuveer temple Jankikund Chitrakoot Satna, Madhya Pradesh where it grows enormously. The plant species is identified by its morphological features and confirmed by Dr. R.L.S. Sikarwar Taxonomic head of Deendayal Research Institute Chitrakoot Satna Madhya Pradesh, India. The plant material was washed with tap water two to three-time. The specimen voucher has been submitted to the Department of Botany, Kalinga University, and Naya Raipur for future reference (Specimen voucher No- APKU4).

Preparation of plant extract

Dried leaves were ground in the mixer and sieved with a 355µm IS sieve. This fine plant powder is used for Physico-chemical analysis. For extraction of plant extract, coarse powder has been prepared and loaded in an extraction chamber of the Soxhlet apparatus. Ethanol is used as a solvent

Physicochemical analysis

An array of physicochemical tests was conducted on plant powder, including the determination of alcohol-soluble extractives, water-soluble extractives, total ash, acid-insoluble ash, loss on drying, and pH determinations (23,24,25).

Determination of PH range

Using a standard glass electrode pH meter, the pH levels of various formulations containing water-soluble portions of *Ficus virens Aiton* leaf powder were measured at concentrations of 1% w/v (1g; 100 ml) and 10% w/v (10 g; 100 ml).

Determination of moisture content

To determine the loss on drying, approximately 1.0 g of *Ficus virens Aiton* leaf powder was placed on a precisely weighed moisture disc (measured using an electronic scale - Dawner). The sample was then dried in an oven at 105 ºC for 3 hours, followed by cooling in a desiccator for 30 minutes before being weighed without delay. The weight loss was calculated as a percentage of the air-dried material.

Extractive Value

To determine the solubility of the extract, the sample was dissolved in different solvents, including water and ethanol.

Determination of total ash

An accurately weighed and previously ignited crucible was used to hold 2 grams of *Ficus virens Aiton* leaves powder. The dried powder was evenly spread in the crucible and ignited gradually by increasing the temperature to 450 °C for 5 hours in a muffle furnace until it turned white, indicating the absence of carbon. After cooling in a desiccator, the crucible was weighed, and the total ash content was determined in milligrams per gram of air-dried material.

Acid Insoluble Ash Value

After adding 25 mL of dilute HCl to the crucible containing total ash, the insoluble matter was collected on an ashless filter paper (Whatman number 41) and washed with hot water until the filtrate reached neutrality. The filter paper, with the insoluble matter, was then transferred to the original crucible, dried on a hot plate, and ignited until a constant weight was obtained. The residue was allowed to cool in a desiccator for 30 minutes before being weighed

promptly. The content of insoluble ash was calculated based on the air-dried plant material.

Phytochemical analysis: Qualitative

Plant extracts were subjected to qualitative chemical tests. The phytochemical screenings included tests for alkaloids, tannins, steroids, glycosides, flavonoids, saponins, carbohydrates, terpenoids, and proteins (23-25).

Test for Alkaloids:

To test for the presence of alkaloids in the leaves extract, approximately 15 mg of the extract was mixed with 6 ml of 1% HCL and stirred in a water bath for 5 to 6 minutes. The resulting solution was then divided equally into three parts.

1. One of the portions was subjected to Dragendorff's test by adding 1 ml of Dragendorff's reagent to 2 ml of the solution. The formation of an orange-coloured precipitate indicated the presence of alkaloids.
2. To perform Mayer's test, 1 ml of Mayer's reagent was added to a portion of the solution (2 ml), and the appearance of a cream-coloured precipitate indicated the presence of alkaloids.
3. The above solution was subjected to Wagner's test by adding a few drops of Wagner's reagent. The formation of a brown precipitate indicates the presence of alkaloids.

Test for Terpenoids (Salkowski test)

A reddish-brown colour indicates the presence of terpenoids when 100 mg of the extract is mixed with 2 ml of chloroform and 2 ml of concentrated H_2SO_4 added along the side of the test tube.

Test for Steroid

Around 1 mL of the crude extract was added to 10 mL of chloroform and 10 mL of sulphuric acid. The appearance of a bilayer, with a red upper layer and a greenish lower layer, confirms the presence of steroids.

Test for Tannins

A mixture was prepared by adding 0.5 g of leaf extract to 10 ml of distilled water, followed by the addition of a few drops of 5% ferric chloride. The presence of tannins is indicated by the formation of a black or blue-green precipitate.

Test for Saponins

The presence of saponins in 0.5 g of leaf extract was determined by shaking it with 10 ml of distilled water in a test tube and warming it in a water bath for 5 minutes. If foaming occurred, it indicated the presence of saponins.

Test for Glycoside

Anthraquinone glycoside (Born Trager's test)

The extract solution (1 ml) was mixed with 1 ml of 5% H_2SO_4 and boiled in a water bath, followed by filtration. Chloroform (equal volume to the filtrate) was added, and the mixture was allowed to stand for 5 minutes. Then, half of its volume of dilute ammonia was added. The appearance of a rose-pink to red colour in the ammonium layer indicates the presence of anthraquinone glycosides.

Cardiac glycoside (Keller-Killiani test)

A mixture of 0.5 g of extract and 5 ml of distilled water was prepared, followed by the addition of 2 ml of acetic acid, some ferric chloride, and 1 ml of H_2SO_4 along the side of the test tube. Synthesis of cardiac glycoside is indicated by the appearance of a brown ring at the interface, followed by a violet ring.

Baljet test

Mix 2 mL of the extract with 1 mL of Baljet reagent (prepared by combining 95 mL of 1% picric acid and 5 mL of 10% NaOH). The appearance of an orange-yellow colour indicates the presence of cardiac glycosides (76).

Phytochemical analysis: Quantitative

Total Phenolic Content (TPC)

For the determination of TPC, the Folin-Ciocalteu method was applied using a spectrophotometer with some modification (Labtronics-2201) as first described by Singleton & Rossi in 1965 (26). First of all 1 mg/ml concentration solution of the plant extract was prepared. Now transfer 1 mL of this solution to a 25 ml volumetric flask. Then 9 ml of distilled water was added to this solution. Then 1ml Folin-Ciocalteu reagent was added to this solution and stirred well for five minutes and then 10 ml of 7% sodium carbonate. Finally, the final volume was made to 25 ml by adding distilled water. Similarly, different concentrations of standard solutions of Gallic acid 20, 40, 60, 80, and 100 µg/ml were prepared and absorbance was noted against the reagent blank at 750 nm on a UV-vis spectrophotometer after 90 minutes of incubation (27-30).

Total Tannin Content (TTC)

To estimate the total tannin content in plant extract, the Folin-Ciocalteu method was used (31-32). First of all, 0.1 ml of the plant extract was transferred to 10 ml of the volumetric flask. Then 7.5ml of distilled water was added to this solution. Now 0.5 ml of Folin-Ciocalteu reagent was added to this solution. After 5 minutes, 1 ml of 35 % Sodium carbonate was added and the mixture was shaken. A set of standard solutions of Gallic acid (20, 40, 60, 80, and 100 µg/ml) was prepared in the same manner. The absorbance of the sample and standard solutions were taken at 725 nm with the help of a UV-visible spectrophotometer against the reagent blank after 30 minutes of the incubation period.

Total Flavonoid Content (TFC)

By using a spectrophotometer aluminium chloride assay, the total flavonoid content of leaf extract has been determined. 1 ml of plant extract has been taken in the 10 ml of the volumetric flask for this experiment. Further, we added 0.3 ml of 5% $NaNO_2$ after adding 4 ml of distilled water and shaking well.

After five minutes, we added 0.3 ml of 10% $AlCl_3$ and 2 ml of 1M NaOH. Double distilled water was added to this mixture to make the final volume 10 ml. By following the same manner set of standards drug quercetin (20, 40, 60, 80, and 100 µg/ml) has been prepared. Using a UV-visible spectrophotometer model LT-2201, absorbance measurements for the standard and test solutions were made at 510 nm after the 30-minute incubation period (33-34).

Anti-inflammatory activity: Human Red Blood Cell (HRBC) method

Preparation of Human Red Blood Cell (HRBC) Suspension

Fresh human blood has been ejected through a syringe from a volunteer. The detail and purpose of the studies have been told to the volunteer and written consent has been taken from the participant. 2 ml of human blood has been mixed with equal volume sterile Alsever solution (2 % dextrose, 0.8 % sodium citrate, 0.05% citric acid and 0.42 % sodium chloride is dissolved in 100ml of water). Centrifuge the mixture for 10 minutes at 3000 rpm, packed cells were washed three times with isosaline. The measured blood volume was used to create a 10% v/v isosaline solution.

Heat-induced Haemolysis

According to this method, membrane lysis was caused when the human blood cell membrane was induced with a hypotonic solution. If the extract/drug can stabilise the HRBC membrane, haemolysis can be prevented. First of all 1 ml of phosphate buffer (pH 7.4, 0.15M), has been taken in a test tube. Added 2 ml of hypo saline (0.36%), and 0.5 ml of HRBC suspension (10% v/v) then add 0.5 ml of plant extracts and various concentrations of the reference drug diclofenac sodium (50, 100, 250, 500, 1000, and 2000 µg/ml) in different test tubes. The percentage of haemolysis was determined using a spectrophotometer with a 560 nm setting. (35-37).

The percentage of Haemolysis of RBC was calculated by using the following formula:

$$\text{The Percentage of Hemolysis} = \frac{\text{Absorbance of test sample}}{\text{Absorbance of Control}} \text{ X } 100$$

The percentage of HRBC membrane stabilisation can be calculated as follows:

$$\text{The Percentage of HRBC Membrane Stabilization} = 100 - \left[\frac{\text{Absorbance of test sample}}{\text{Absorbance of Control}} \text{ X } 100\right]$$

Free Radical Scavenging Activity (DPPH assay)

The DPPH method was used to assess the ability of the ethanolic extract of *Ficus virens Aiton* leaves to scavenge free radicals. It has been checked if the plant sample scavenges coloured α, α-diphenyl-β-picrylhydrazyl (DPPH) which colour fades after scavenging. Standard DPPH solution prepared by dissolving the crystal of DPPH) (1.3 mg/ml in methanol). 10 ml of methanol have been used to dissolve 10 mg of plant extract. One ml of this solution was placed in test tubes and diluted up to ten times using the same solvent. Stock solutions with concentrations of 10, 15, 25, 50, and 60 µg/ml were obtained and placed in different test tubes in amounts of 0.10, 0.15, 0.25, 0.50, and 0.60 ml. Make the final volume up to 1 ml by adding the same solvent. 75µl of freshly prepared DPPH was added to all test tubes. The absorbance was measured at 517 nm using a Labtronics UV-visible spectrophotometer (model no. 2201). A set of ascorbic acids dissolved in water with the same concentration was used to create a control sample. The following equation was used to determine the percentage of radical scavenging activity in test and standard samples. Each experiment was carried out three times. The percentage of inhibition by different concentrations of test samples and standards was calculated by the following equation (38-40).

$$\text{The Percentage of Inhibition} = \frac{\text{A control} - \text{A sample}}{\text{A control}} \text{X} 100$$

A control = Absorbance of DPPH reagent alone
A sample = Absorbance of DPPH reagent along with different concentrations of extracts.

Anti-sickling activity (Emmel Test)

Sickle cell blood has been collected from the 14-year-old patient whose treatment is going in Sickle cell institute Raipur. Written consent has been taken from her parents. Sickle cell blood was diluted with 150mM phosphate-buffered saline (NaH_2PO_4 30mM, Na_2HPO_4 120mM, NaCl 150mM) and mixed with an equivalent volume of 2% sodium metabisulphite ($Na_2S_2O_5$). A drop from the mixture was spotted on a microscope slide in the presence or absence of anthocyanins extracts and covered with a cover slip. Paraffin was applied to seal the edges of the cover completely to exclude air (Hypoxia). (41-42)

The anti-sickling activity of plant extract has been evaluated through a reversal test. Firstly all the erythrocytes were reduced by using sodium metabisulphite and incubated for 2 hours. After two hours cells were examined under the microscope and the percentage of sickling was calculated by using the following formula.

$$\text{The percentage of sickling} = \frac{Number\ of\ sickling}{Total\ number\ of\ cell} \text{ x } 100$$

If the sickling percentage is below 100 %, the time had been increased accordingly to obtain 100% sickling. In our experiment, a 100% sickling rate was obtained after 2 hours of incubation.

Reversibility Test

Different concentrations of plant extracts starting from 50 µg/ml to 10 mg/ml had been prepared in saline solution (0.86% NaCl) and tested against sickle cells at different time intervals. One hundred fifty microliters of plant extract had been added to the 150µl of the previous solution (previously 2 hours incubated blood with sodium metabisulphite). The same procedure was done with the positive control (phenylalanine) and the negative control.

https://doi.org/10.47552/ijam.v16i1.5317 ISSN No: 0976-5921

International Journal of Ayurvedic Medicine, Vol 16 (1), 2025; 52-63

The change in the percentage of residual sickle cells over time was given by the following relationship

$$\text{Percentage of residual sickle cell} = \frac{\textit{Sickle cell average at Tx}}{\textit{Sickle cell average at T0}}$$

T_x= 0, 30, 60, 90, 120, and 150 minutes
T_0 = temperature initial
The percentage of reversibility of sickle cell was calculated by the given formula

$$\frac{\text{Total number of normal cells}}{\text{Total number of sickled cells}} \text{ X } 100$$

Result and Discussion

Morphology and anatomy of the plant: Ficus virens Aiton (Hindi Name- Plaksha, English Name- White Fig)

Ficus virens Aiton is a large spreading tree like other plants of the Fabaceae family. The plant stem was shown in Figure 1 (a). Leaves are petiolate; alternate; simple; stipulate as it is shown in figure 1 (b). The inflorescence is catkin type. The flower is small inconspicuous, bracteates and bracteolate, actinomorphic, incomplete, unisexual, and hypogynous. The plant contains four perianths which are arranged in two whorls. Stamens are present in four to five numbers. Male flowers are opposite to the perianth. Tow carpel is present in female flowers which are simple, the ovary is superior type, and apocarpous and basal placentation is found. Leaf and stem sections were cut out by free-hand sectioning using a blade. The transverse section of the leaf and stem show the epidermis, cortex, and endodermis. The vascular bundle is endarch, collateral, and conjoint as it is shown in Figure 2. (a), (b) and SEM image of the plant stem shown in Figure 2 (c)

Leaves are petiolate and green in colour. At immaturity, they are reddish-brown in colour but when they mature they acquire green colour. Leaves are simple alternate spirals. Aerial roots are also present to support the main trunk of the plant body but they are not as hard as the banyan tree. Aerial roots are attached to the main stem and give strength to the main stem. The milky latex is also present which is very nutritious in nature.

They attract lots of insects and ants during bleeding and injury of the plant portion. Leaves of *Ficus virens Aiton* start from 8cm to 19cm long and 3 to 6 cm wide. The midrib is whitish. Stipules are less than 1cm long. It is a deciduous tree. Plant shades their leaves in February and new leaves emerge in March with colours of purple red and bronze, giving the tree a wonderful look. The colour transformation goes on till April. It acquires a height up to 30 meters. Flowers unisexual; inflorescence syconia, axillary, paired, 1-1.5 cm across, globose, often obconical; peduncle 1-6 mm long, slender, pubescent; basal bracts 3, ovate, acute, persistent; orifice plane, closed by 3 flat apical bracts in a disc 1 mm wide, internal bristles abundant, white, chaffy-vesicular; flowers of 4 kinds; male flowers ostiolar, sessile, in 2-3 rings; tepals 2-3, ovate, acute or shortly gamophyllous, stamen 1, filament 0.5 mm; anther oblong, parallel; female flowers sessile; tepals 3-4, free, ovary superior, 1.2 mm, obovoid, sessile or stalked, red-brown, style filiform, tapering, gall flowers sessile or shortly pedicellate, tepals 3-4, reddish, spathulate to linear-lanceolate, free, ovary sessile or stalked, red-brown.

Figure 1: (a) Stem of *Ficus virens Aiton* and (b) leaves of *Ficus virens Aiton*

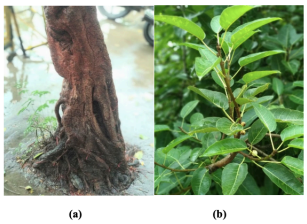


Figure 2(a): T.S. of the leaf of *Ficus virens Aiton*

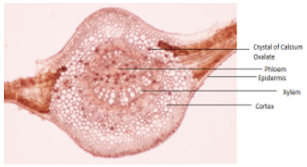


Figure 2(b): T.S. of stem of *Ficus virens Aiton (Pr- periderm, Cr- cortex, Ph- phloem, Xy- xylem, Hy-Hypodermis)*

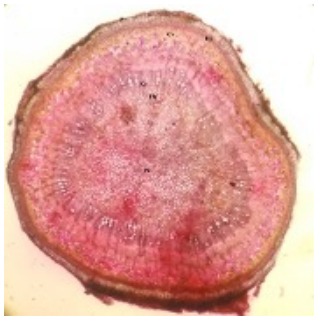
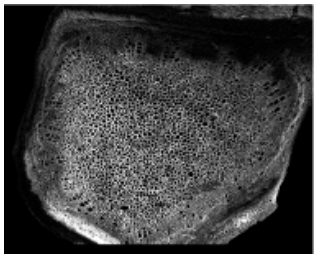


Figure 2(c): SEM Image of *Ficus virens Aiton* stem



Physico-chemical analysis

The powdered drug was evaluated for its physicochemical analysis which includes a loss on drying total ash, acid insoluble ash, and water-soluble ash. The analysis of the physical and chemical properties of plant drugs is crucial for identifying impurities and mishandling during their preparation. A high amount of total ash can indicate improper handling or the presence of inorganic impurities, while acid-insoluble ash may suggest contamination with materials such as silica, earth, or sand. Comparing acid-insoluble ash with the total ash value of the same sample can help differentiate between natural ash variations and contaminants. Water-soluble ash represents the portion of the total ash that dissolves in water and is a useful indicator of the presence of water-soluble salts in the drug. Extractive values reflect the presence of polar or nonpolar compounds that can be extracted from plant material. Removing moisture from crude drugs is essential to prevent spoilage by bacteria and molds, as well as to avoid enzymatic destruction of active ingredients (43). The finding of all these parameters is given in Table 1.

Table 1: Physico-chemical analysis of plant

Parameters	Outcome
Loss on drying (LOD) %	7.63±1.01
Solubility on water %	7.28±0.93
Solubility in Ethanol %	7.33±0.85
Total Ash %	11.46±1.23
Acid Ash Insolubility %	2.04±0.53
PH of 1% w/v formulation solution	6.5

Phytochemical analysis of plant extract

Preliminary phytochemical testing of the plants revealed the presence or absence of alkaloids, terpenoids, tannins, reducing sugar, saponins, and glycoside. Phytochemical screening is a useful tool that not only identifies the various constituents present in plant extracts but also determines which ones are present in higher quantities. Additionally, this screening process can aid in the discovery of bioactive compounds that have the potential to be used in the development of new and effective drugs (44). Previous research showed that alkaloids contain anti-inflammatory (45), antimalarial (46), and antimicrobial activities (47). Similarly, the presence of steroids has demonstrated that plants should have insecticidal and antibacterial properties (48). Other secondary metabolites such as cardiac glycosides are used to treat congestive heart failure and cardiac arrhythmia (49). Table 2 shows the presence or absenteeism of phytochemicals in *Ficus virens Aiton.*

Table 2: Phytochemical screening of the *Ficus virens Aiton*

Test	*Presence (+), Absence* (-)
Alkaloids	
(a) Dragendorff's test	+
(b) Mayer's Test	+
(c) Wagner's Test	+
Terpenoids	
Salkowski Test	+
Steroid Test	+
Tannin	+
Reducing sugar	+
Saponins	+
Glycoside	
(a) Anthraquinone Glycoside	-
(b) Cardiac Glycoside (Baljet test)	+
(c) Keller-Killiani test	-

Total Phenolic Contents (TPC)

Total phenolic content in terms of equivalent to Gallic acid was quantified per µg/mg of plant extract. By using the standard curve and equation derived from the graph i.e. y = 0.0026x - 0.0026, R^2 = 0.9981 (figure 3), the values of the total phenolic content of the test sample have been calculated. Total phenolic content (TPC) is recorded at 79.84±2.77 µg/mg of GAE (Gallic acid equivalent). Previous research showed that the methanol fraction of *F. virens* contained a phenolic content of 1267.35 mg GAE/g dry extract (50). Another study conducted in Egypt found TPC only 2.50±0.715 mg/ gram of Gallic acid equivalent (51). Redox

Figure 3: Standard curve of Gallic acid for total phenolic content

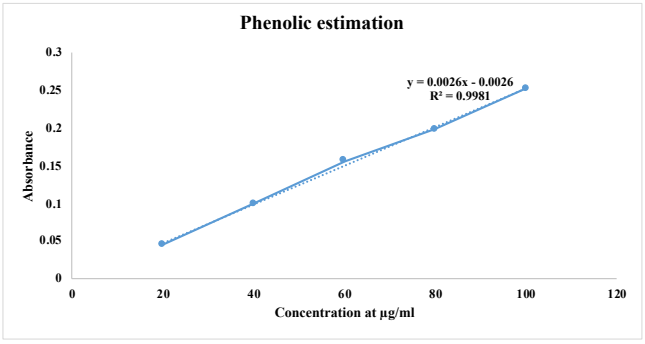


Figure 4: Standard curve of Gallic acid for total tannin content

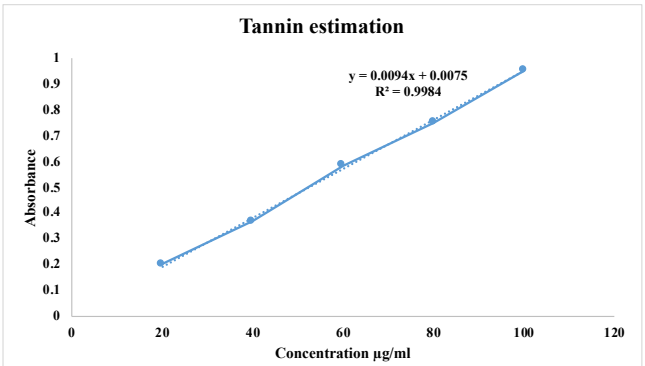
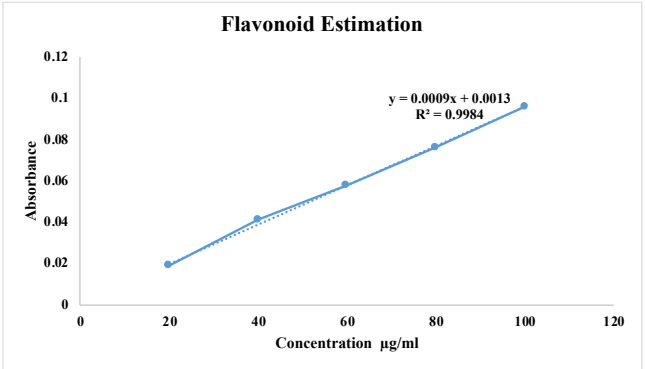


Figure 5: Standard curve of quercetin for total Flavonoid Content



properties attributed to phenolic compounds, which are significant constituents of plants, play a crucial role in their antioxidant activity (52).

Total Tannin Contents (TTC)

The total tannin content of the leaves of *Ficus virens Aiton* was calculated by using the formula y = 0.0094x + 0.0075, R^2 = 0.9984 (figure 4) extracted from the standard curve of Gallic acid for tannin estimation. The value of total tannic contents was expressed in terms of µg of per mg of Gallic acid equivalent. Leaves of ethanolic extract of *Ficus virens Aiton* showed 10.51±0.63 µg/mg of Gallic acid equivalent. Numerous studies have demonstrated the antioxidant potential of major natural product constituents such as polyphenols, terpenes, and polyunsaturated fatty acids (53, 54)

https://doi.org/10.47552/ijam.v16i1.5317 ISSN No: 0976-5921

International Journal of Ayurvedic Medicine, Vol 16 (1), 2025; 52-63

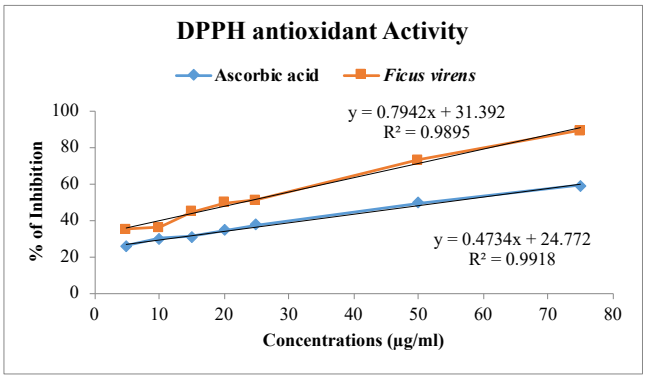
Total Flavonoid Contents (TFC)

The total flavonoid content of the plant part was calculated by using the formula y = 0.0009x + 0.0013, R^2 = 0.9984 (figure 5). The values of TFC are expressed in terms of µg per mg of the equivalent of quercetin. The values of TFC were recorded at 132.63±6.70 µg/ mg of quercetin equivalent (QE). Previous research showed that the methanolic extract of leaves of *F. virens* contained the maximum amount of flavonoids than other *Ficus* species such as *Ficus benghalensis, Ficus religiosa, Ficus elastica*. (1080.61 mg QE/g dry extract) (50). Secondary metabolites known as flavonoids exhibit antioxidant properties, and the effectiveness of their antioxidant activity is influenced by the quantity and arrangement of unbound hydroxyl groups (55).

Antioxidant activity of *Ficus virens Aiton*

DPPH is a free radical with an unpaired electron located on nitrogen, which exhibits a robust absorption peak at 517 nm. Its colour changes from purple to yellow when it undergoes electron-pairing with a radical scavenger, leading to the formation of reduced DPPH-H (56). The ethanolic extract of *Ficus virens Aiton* (EEFV) showed strong antioxidant potential in the DPPH method as shown in Figure 6. At 75 µg/ml, the highest antioxidant activity has been recorded it will scavenge 89.34±0.56% of DPPH at the same concentrations as ascorbic acid scavenges 59.13±0.98% so it will be quite better than ascorbic acid. IC_{50} of EEFV is observed at 22.76±1.01 µg/ml so it is almost half of the IC_{50} of ascorbic acid which is 51.88±0.43 µg/ml. The percentage of inhibition of DPPH by different concentrations of the standard drug was given in Table 3 and the inhibition of DPPH by EEFV has been given in Table 4. A previous study showed that the

Figure 6: Antioxidant potential of *Ficus virens Aiton* with the comparison of Ascorbic acid through DPPH assay



antioxidant activity of *Ficus virens Aiton* leaf extract significantly is dependent on concentration. Young leaf extract of *F. virens var. sublanceolata* inhibits 97.68% ABTS radical at 0.5 mg/ml of extract concentration. *F. virens var. sublanceolata* and, *F. virens var. verins* required 0.34 mg/ml and 1.03mg/ml to scavenge 50% of the DPPH (57). Earlier reports have demonstrated scavenging activities of ethanol extract of *F. carica* L.

Table 3: Percentage of inhibition of DPPH by different concentrations of Ascorbic acid

Concentrations (µg/ml)	*First reading*	*Second reading*	*Third reading*	*Mean*
5	26.1	24.03	27.32	25.82±1.66
10	28.72	33.02	28.56	30.10±2.53
15	30.05	31.15	32.04	31.08±1.00
20	36.1	32.35	35.12	34.52±1.94
25	39.45	36.15	37.38	37.66±1.67
50	52.15	47.05	50.12	49.77±2.57
75	61.35	56.02	60.04	59.14±2.78

Table 4: Percentage of inhibition of DPPH by ethanolic extract of *Ficus virens Aiton*

Concentrations	*First reading*	*Second reading*	*Third reading*	*Mean*
5µg/ml	36.8	34.56	34.23	35.20±1.40
10 µg/ml	37.2	35.46	34.97	35.88±1.17
15 µg/ml	46.53	43.64	43.85	44.67±1.61
20 µg/ml	50.15	48.14	50.04	49.44±1.13
25 µg/ml	52.35	50.85	49.68	50.96±1.34
50 µg/ml	75.25	72.87	71.2	73.11±2.04
75 µg/ml	90.56	88.21	89.25	89.34±1.18

fruits and the methanol extract of *F. microcarpa* L. fil. leaves against ABTS+ and DPPH free radicals (58, 59).

The value of inhibition of DPPH by EEFV has been calculated by using the formula (y = 0.7942x + 31.392) and the value of standard drug inhibition was calculated by the given formula (y = 0.4734x + 24.772).

The present study shows leaf extract contains significant antioxidant activity in comparison to ascorbic acid. A lower IC_{50} value indicates higher antioxidant potential. The result also showed that plant contains high amounts of flavonoid and phenolic compounds which contributed to enhancing the antioxidant potential of plant parts. The redox properties of phenolic compounds are responsible for their antioxidant activity, which plays a crucial role in absorbing and neutralising free radicals, as well as quenching singlet and triplet oxygen. Additionally, phenolic compounds possess the ability to chelate metals (60).

Anti-inflammatory activity of *Ficus virens Aiton*

Ficus virens Aiton show significant anti-inflammatory activity and an almost similar response to diclofenac sodium. At 50 µg/ml of the extract, 55.51±2.63 % of haemolysis was observed which was further reduced up to 12.19±1.84 % at 2000 µg/ml while in the case of diclofenac sodium; 45.25±1.41 % of haemolysis was recorded at 50 µg/ml which was further reduced up to 9.57±2.03 at 2000 µg/ml of concentrations. Figure 5 shows the reduction trend of haemolysis on adding the concentrations while Figure 8 shows the percentage of protection. Table 5 shows the haemolysis reduction data of ethanolic extract of *Ficus virens Aiton* (EEFV) and standard drug diclofenac sodium.

https://doi.org/10.47552/ijam.v16i1.5317 ISSN No: 0976-5921

Figure 7: Haemolysis inhibition of HRBC through diclofenac sodium and ethanolic extract of *Ficus virens Aiton* (EEFV)

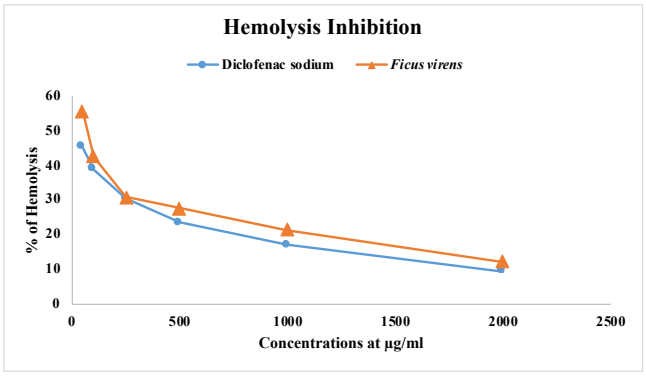


Figure 8: Protection of lysis of HRBC in the presence of diclofenac sodium and ethanolic extract of the *Ficus virens Aiton*

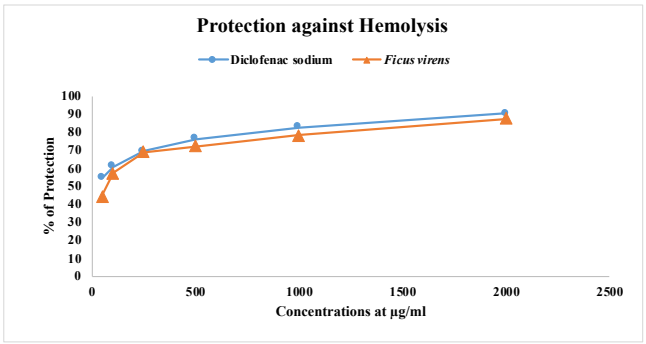


Table 5: Percentage of haemolysis inhibition done by EEFV and Standard drug Diclofenac sodium

Concentrations	Ethanolic extract of the *Ficus virens Aiton*	Standard drug (Diclofenac Sodium)
50 µg/ml	55.51±2.63	45.25±1.41
100 µg/ml	42.48±157	38.76±2.61
250 µg/ml	30.75±2.34	30.25±1.05
500 µg/ml	27.65±3.27	23.43±1.54
1000 µg/ml	21.5±2.19	17.12±2.36
2000 µg/ml	12.19±1.84	9.57±2.03

The anti-inflammatory activity of plant extract is evaluated through the HRBC suspension method. The erythrocyte membrane is similar in structure to the lysosomal membrane, the stabilisation of the former suggests that the extract could potentially stabilise lysosomal membranes as well (61). Preventing the release of lysosomal constituents, such as bactericidal enzymes and proteases, from activated neutrophils is crucial for limiting the inflammatory response and reducing tissue inflammation and damage caused by their extracellular release. Thus, the stabilisation of the lysosomal membrane plays a vital role in achieving this goal (62). While the precise mechanism by which the extract stabilises the membrane is still unknown, hypotonicity-induced haemolysis is thought to result from the shrinking of cells due to the loss of intracellular electrolytes and fluid components through osmosis. The extract could potentially inhibit the

Figure 9: Percentage of the reversion rate in the presence of the ethanolic extract of *Ficus virens Aiton* at concentrations of 50 µg/ml

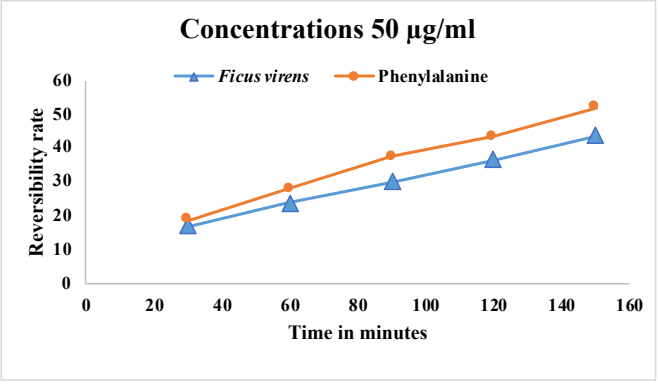


Figure 10: Percentage of the reversion rate in the presence of the ethanolic extract of *Ficus virens Aiton* at concentrations of 500 µg/ml

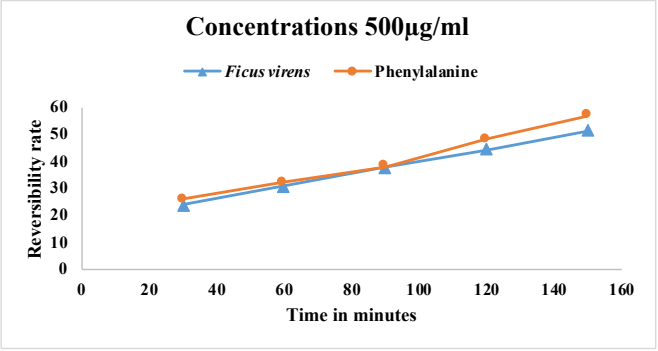


Figure 11: Percentage of the reversion rate in the presence of the ethanolic extract of *Ficus virens Aiton* at concentrations of 5 mg/ml

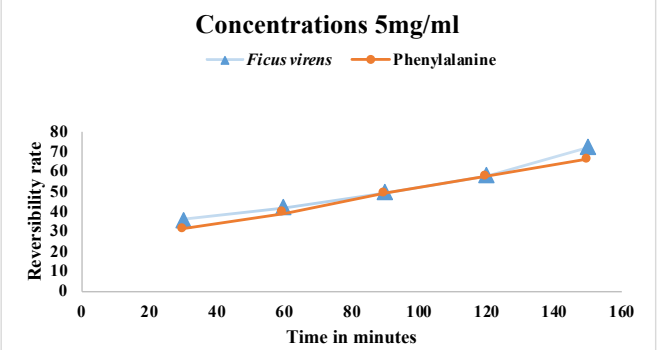
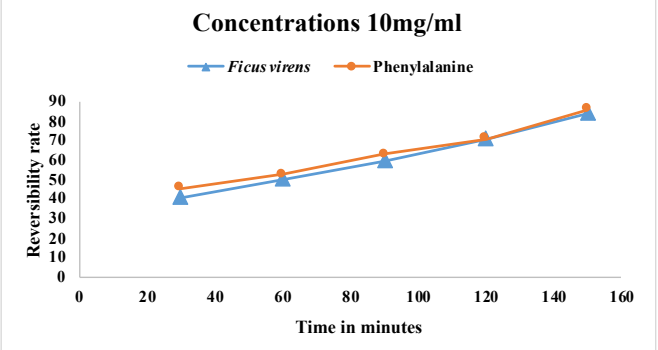


Figure 12: Percentage of the reversion rate in the presence of the ethanolic extract of *Ficus virens Aiton* at concentrations of 10 mg/ml.



processes that stimulate or facilitate the efflux of these intracellular components (63, 64).

Anti-sickling activity *of Ficus virens Aiton*

Figures 9 to 12 show the anti-sickling activity of plant extract. The anti-sickling activity of *Ficus virens Aiton* has shown a significant reversal rate of sickle cell. The extract showed almost equal potential to phenylalanine. As the concentrations increased, the reversibility rate also increased in the same manner. At 50 µg/ml of concentrations of plant extract 43.62± 1.00 reversal rate has been recorded after 150 minutes of the incubation period. The anti-sickling activity of plant extract increased with the enhancement of concentrations. The reversal rate has been increased when we treat sickle cell with 500 µg/ml and goes up to 51.31±1.50%. When the concentrations increase ten times more it will show a 72.48±0.50 reversal rate and its further increase reached up to 84.13±0.25 % when sickle cells are treated with 10 mg/ml of concentrations and 150 minutes of the incubation period. All the values of reversibility of sickle cells at different concentrations and time periods are given in Table 6.

Table 6: Percentage of reversal of sickle cells at different concentrations of *Ficus virens Aiton* and Phenylalanine at different time intervals

Time in Minutes	Fv* 50 µg/ ml	Pa* 50 µg/ml	Fv 500 µg/ ml	Pa 500 µg/ ml	Fv 5 mg/ml	Pa 5mg/ml	Fv 10 mg/ ml	Pa 10 mg/ ml
30	16.89±0.65	18.67±1.34	23.88±1.65	25.93±1.76	35.83±1.78	31.17±0.78	40.88±2.00	45.95±1.92
60	23.7±.085	28.00±156	30.97±1.35	31.48±137	41.98±1.24	39.61±0.89	50.38±1.75	52.60±1.76
90	29.93±1.25	37.33±1.96	37.75±0.85	38.27±1.64	50.00±1.55	49.35±1.36	59.86±1.37	62.99±1.32
120	36.69±1.67	43.33±1.38	44.30±1.15	48.15±2.04	58.45±1.65	57.79±1.45	70.92±0.65	70.78±1.54
150	43.62±1.00	52.00±1.69	51.31±1.50	56.79±2.17	72.48±0.50	66.23±1.04	84.14±0.25	85.71±1.14

*Fv: *Ficus virens Aiton*, Pa: Phenylalanine

The result of this study reported that plant extracts contain high amounts of phenolic and flavonoid content. In previous research, various flavonoids reported in plant extract including quercetin-3-O-α-D-arabinopyranoside, quercetin-3-O-β-D-galactopyranoside, kaempferol-3-O-α-Darabinopyranoside, and kaempferol-3-O-β-D-galactopyranoside (65). In addition to this, many phenolic compounds are also reported in the plant extract. It has been previously reported in the literature that flavonoids may act as potent anti-sickling agents. The flavonoids present in the extract may be responsible for the plant's anti-sickling potential (66, 67).

The phytochemical screening of extracts showed the presence of different secondary metabolites such as phenols, flavonoids, alkaloids, and saponins. Extracts might have anti-sickling properties due to an inhibitory effect on in vitro haemoglobin polymerization or structural modifications linked to haemoglobin's environment (68).

Biomolecules' ability to avoid in vitro polymerization is determined by one or more of the following factors: (a) Their tendency and efficiency to bind to the complementary contact region/site of deoxyHbS monomers (68, 69); (b) Modification of the HbS contact region by amino acid residues (70); (c) Stabilization of the R state of HbS molecules (71, 72, 70). In addition to inhibiting sickle cell haemoglobin polymerization, antioxidant molecules improved oxidant status in sickle erythrocytes in studies (73-75)

Conclusion

The study showed that plant extract contains significant antioxidant, anti-inflammatory, and anti-sickling potential. Many more in-vivo and clinical studies are required to confirm its effectiveness against these ailments. If the result will be promising in the clinical study, then the herbal drug can be formed using the plant extract. This will not only replace the NSAIDs and hydroxyurea but also attract more plant-based studies to treat different ailments and diseases. Future research also can be focused and more centric to find out the therapeutic molecules present in the extract. Moreover, it is necessary to conduct toxicity profiling and bioguided fractionation studies

Conflict of interest statement

The authors declare there was no conflict of interest.

Acknowledgement

The authors express their sincere gratitude to the higher authorities of Kalinga University, for providing the essential resources for this study. The authors would also like to acknowledge Mr. Dulendra Sahu, lab attendant, Department of Botany, for his cooperation and support during the study.

Funding

The authors have not received extramural support/funds for the study.

References

1. Serjeant GR. Serjeant, BE. Homozygous sickle cell disease In Sickle cell disease. 3rd ed. New York: Oxford University Press; 2001. 429-35.
2. Pauling L, Itano HA, Singer SJ, Wells IC. Sickle cell anaemia is a molecular disease. *Science*. 1949; 110(2865): 543-548. DOI: 10.1126/ science.110.2865.543
3. Ingram VM. Gene mutations in human haemoglobin: the chemical difference between

normal and sickle cell haemoglobin. *Nature*. 1957; 180(4581): 326-8. DOI: 10.1038/180326a0.
4. Eaton WA, Hofrichter J. Hemoglobin S gelation and sickle cell disease. *Blood*. 1987; 70(5): 1245-6.
5. Colah RB, Mukherjee MB, Martin S, Ghosh K. Sickle cell disease in tribal populations in India. *Indian J Med Res*. 2015; 141(5): 509-15. DOI: 10.4103/0971-5916.159492.
6. Mehanna AS. Sickle cell anaemia and antisickling agents then and now. *Curr Med Chem*. 2001; 8(2): 79-88. DOI: 10.2174/0929867013373778.
7. Hoppe CC. Inflammatory mediators of endothelial injury in sickle cell disease. *Hematology/Oncology Clinics*. 2014; 28(2):265-86.
8. Mantovani A, Cassatella MA, Costantini C, Jaillon S. Neutrophils in the activation and regulation of innate and adaptive immunity. *Nat. Rev. Immunol*. 2011; 11(8):519-31.
9. Ortega-Gómez A, Perretti M, Soehnlein O. Resolution of inflammation: an integrated view. *EMBO molecular medicine*.2013; 5(5): 661-74.
10. Hebbel RP. Ischemia-reperfusion injury in sickle cell anaemia: relationship to acute chest syndrome, endothelial dysfunction, arterial vasculopathy, and inflammatory pain. *Hematology/oncology Clinics*. 2014; 28(2):181-98.
11. Zhang D, Xu C, Manwani D, Frenette PS. Neutrophils, platelets, and inflammatory pathways at the nexus of sickle cell disease pathophysiology. Blood, *The Journal of the American Society of Hematology*. 2016; 27(7): 801-9.
12. Lanaro C, Franco-Penteado CF, Albuqueque DM, Saad ST, Conran N, Costa FF. Altered levels of cytokines and inflammatory mediators in plasma and leukocytes of sickle cell anaemia patients and effects of hydroxyurea therapy. *Journal of leukocyte biology*. 2009; 85(2): 235-42.
13. Qari MH, Dier U, Mousa SA. Biomarkers of inflammation, growth factor, and coagulation activation in patients with sickle cell disease. Clinical and Applied Thrombosis/Hemostasis.2012; 18(2):195-200.
14. Sarray S, Saleh LR, Saldanha FL, Al-Habboubi HH, Mahdi N, Almawi WY. Serum IL-6, IL-10, and TNFα levels in pediatric sickle cell disease patients during vasoocclusive crisis and steady state condition. Cytokine. 2015; 72(1): 43-7.
15. Conran N, Franco-Penteado CF, Costa FF. Newer aspects of the pathophysiology of sickle cell disease vaso-occlusion. Hemoglobin. 2009; 33(1):1-6.
16. Patra PK, Chauhan VS, Khodiar PK, Dalla AR, Serjeant GR. Screening for the sickle cell gene in Chhattisgarh state, India: an approach to a major public health problem. *Journal of community genetics*. 2011; 2:147-51.
17. Paunipagar PV, Pati SK, Singh CM, Arya RC. Sickle cell gene in the tribal area of Rajnandgaon district of Chhattisgarh. *Indian Journal of Preventive & Social Medicine*. 2006; 37:143-7.
18. Tripathi S, Mishra N, Kumar A. Prevalence of Sickle Cell Anaemia in Association of Plasmodium falciparum from Rajnandgaon, *India. Ann Nutr Disord & Ther* Jan 8(1): id1067 (2021); 8:1067.
19. Khan KY, Khan MJ, Ahmad M, Mazari P, Hussain I, Ali B, Fazal H, Khan IZ. Ethnobotanical Species of genus Ficus L. Used to treat Diabetes in Pakistan. *Journal of Applied Pharmaceutical Science*. 2011; 1(6):209-11.
20. Shi Y, Hu H, Xu Y, Liu A. An ethnobotanical study of the less known wild edible figs (genus Ficus) native to Xishuangbanna, Southwest China. *Journal of ethnobiology and ethnomedicine*. 2014; 10(1):1-1.
21. Orabi MA, Orabi EA. Antiviral and antioxidant activities of flavonoids of *Ficus virens Aiton* : Experimental and theoretical investigations. *Journal of Pharmacognosy and Phytochemistry*. 2016; 5(3):120-8.
22. Chen XX, Lam KH, Chen QX, Leung GP, Tang SC, Sze SC, Xiao JB, Feng F, Wang Y, Zhang KY, Zhang ZJ. *Ficus virens Aiton* proanthocyanidins induced apoptosis in breast cancer cells concomitantly ameliorated 5-fluorouracil-induced intestinal mucositis in rats. *Food and Chemical Toxicology*. 2017; 110:49-61.
23. Lohar DR. Protocol for testing of ayurvedic, siddha and unani medicines. Pharmacopoeial Laboratory for Indian Medicine, AYUSH. Ministry of Health and Family Welfare.
24. Madhav NS, Upadhyaya K, Bisht AS. Phytochemical screening and standardization of polyherbal formulation for dyslipidemia. International *Journal of Pharmacy and Pharmaceutical Sciences*. 2011; 3(3):235-8.
25. Wallis TE. Practical pharmacognosy. Churchill; 1953.
26. Singleton VL, Rossi JA. Colourimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. American journal of Enology and Viticulture. 1965; 16(3): 144-58.
27. Ghasemzadeh A, Jaafar HZ, Rahmat A. Antioxidant activities, total phenolics and flavonoids content in two varieties of Malaysia young ginger (Zingiber officinale Roscoe). Molecules. 2010; 15(6):4324-33.DOI: https://doi.org/10.3390/molecules15064324
28. Rasool N, Rizwan K, Zubair M, Naveed KU, Imran I, Ahmed VU. Antioxidant potential of different extracts and fractions of Catharanthus roseus shoots. *International journal of phytomedicine*. 2011; 3(1):108.
29. Stankovic MS, Niciforovic N, Topuzovic M, Solujic S. Total phenolic content, flavonoid concentrations and antioxidant activity, of the whole plant and plant parts extracts from Teucrium montanum L. var. montanum, f. supinum (L.*) Reichenb. Biotechnology & Biotechnological Equipment*. 2011; 25(1):2222-7.
30. Pandey AK, Pande P. Evaluation of phytochemicals, anti-inflammatory and antioxidant potential of Aegle Marmelos L. leaves. *Advances in Pharmacology and Pharmacy*. 2023; 11(1):66–77. DOI: https://doi.org/10.13189/app.2023.110107

31. Ribarova F, Atanassova M, Marinova D, Ribarova F, Atanassova M. Total phenolics and flavonoids in Bulgarian fruits and vegetables. *JU Chem. Metal*. 2005; 40:255-60.
32. Singh R, Verma PK, Singh G. Total phenolic, flavonoids and tannin contents in different extracts of Artemisia absinthium. *Journal of Complementary Medicine Research*. 1970; 1(2):101.
33. Kaviarasan S, Naik GH, Gangabhagirathi R, Anuradha CV, Priyadarsini KI. In vitro studies on antiradical and antioxidant activities of fenugreek (Trigonella foenum graecum) seeds. *Food chemistry*. 2007; 103(1):31-7.
34. Xu BJ, Chang SK. Total phenolic content and antioxidant properties of eclipse black beans (Phaseolus vulgaris L.) as affected by processing methods. *Journal of food science*. 2008; 73(2):19-27.
35. Yesmin S, Paul A, Naz T, Rahman AB, Akhter SF, Wahed MI, Emran TB, Siddiqui SA. Membrane stabilization as a mechanism of the anti-inflammatory activity of ethanolic root extract of Choi (Piper chaba*). Clinical Phytoscience*. 2020; 6(1):1-0.
36. Parvin MS, Das N, Jahan N, Akhter MA, Nahar L, Islam ME. Evaluation of in vitro anti-inflammatory and antibacterial potential of Crescentia cujete leaves and stem bark. BMC research notes. 2015; 8:1-7.
37. Chippada SC, Volluri SS, Bammidi SR, Vangalapati M. In vitro anti-inflammatory activity of methanolic extract of Centella asiatica by HRBC membrane stabilisation. *Rasayan J Chem*. 2011; 4(2):457-60.
38. Nariya PB, Bhalodia NR, Shukla VJ, Acharya R, Nariya MB. In vitro evaluation of antioxidant activity of Cordia dichotoma (Forst f.) bark. *Ayu*. 2013; 34(1):124.
39. Anandjiwala S, Bagul MS, Parabia M, Rajani M. Evaluation of free radical scavenging activity of an ayurvedic formulation, Panchvalkala. *Indian journal of pharmaceutical sciences*. 2008; 70(1):31.
40. Ravishankara MN, Shrivastava N, Padh H, Rajani M. Evaluation of antioxidant properties of root bark of Hemidesmus indicus R. Br.(Anantmul). Phytomedicine. 2002; 9(2):153-60.
41. Mpiana PT, Mudogo V, Tshibangu DS, Ngbolua KN, Mangwala PK, Atibu EK, Kakule MK, Makelele LK, Bokota MT. "Antisickling Activity and Thermodegradation of an Anthocyanin fraction from Ocimum basilicum L.(LAMIACEAE)." *Comp. Bio. Nat. Products,* 2010; 3:278-87.
42. Kplé TK, Akakpo-Akue J, Golly JK, Fofie Y, Ahon MG, Kra MA, Sanogo I, Yapo-Crezoit AC. "Phytochemical characterization of three plants and their antisickling activity in the management of sickle cell disease". Journal of Biosciences and Medicines. 2020; 8(6):100-12.
43. Mukherjee PK. Quality Control of Herbal Drugs. New Delhi, India: Business Horizons. 2002.
44. Okoli RI, Turay AA, Mensah JK, Aigbe AO. Phytochemical and antimicrobial properties of four herbs from Edo state, *Nigeria. Rep Opin*. 2009; 1:67-73.
45. Souto AL, Tavares JF, Da Silva MS, Diniz MD, de Athayde-Filho PF, Filho JM. Anti-inflammatory activity of alkaloids: an update from 2000 to 2010. *Molecules*. 2011; 16(10): 8515-34.
46. Dua VK, Verma G, Singh B, Rajan A, Bagai U, Agarwal DD, Gupta NC, Kumar S, Rastogi A. Anti-malarial property of steroidal alkaloid conessine isolated from the bark of Holarrhena antidysenterica. *Malaria journal*. 2013; 12(1):1-6.
47. Benbott A, Yahyia A, Belaidi A. Assessment of the antibacterial activity of crude alkaloids extracted from seeds and roots of the plant Peganum harmala L. *Journal of Natural Product and Plant Resources.* 2012; 2(5):568-73.
48. Bagrov AY, Shapiro JI, Fedorova OV. Endogenous cardiotonic steroids: physiology, pharmacology, and novel therapeutic targets. *Pharmacological reviews*. 2009; 61(1):9-38.
49. Kren V, Martínková L. Glycosides in medicine: "The role of glycosidic residue in biological activity". *Current medicinal chemistry.* 2001; 8(11):1303-28.
50. Dutta R, Bhattacharya E, Pramanik A, Hughes TA, Biswas SM. Potent nutraceuticals having antioxidant, DNA damage protecting potential and anti-cancer properties from the leaves of four Ficus species. Biocatalysis and Agricultural Biotechnology. 2022 Sep 1; 44:102461. DOI: https://doi.org/10.1016/j.bcab.2022.102461
51. Ali, Heba & Shihata, Eman & Yehia, Samar & Mohamed, Khaled & Kamel, Mohamed. Quantitative Analysis of Total Phenolic and Total Flavonoid Constituents of some Ficus species.2018
52. Soobrattee, M.A.; Neergheen, V.S.; Luximon-Ramma, A.; Aruoma, O.I.; Bahorun, T. Phenolics as potential antioxidant therapeutic agents: Mechanism and actions. Mutat. *Res.-Fund. Mol. Mutagen*. 2005; 579:200–213.
53. Yin MC, Lin MC, Mong MC, Lin CY. Bioavailability, distribution, and antioxidative effects of selected triterpenes in mice. *Journal of agricultural and food chemistry*. 2012; 60(31):7697-701.
54. Pu, F., Ren, XL. & Zhang, XP. Phenolic compounds and antioxidant activity in fruits of six *Diospyros kaki* genotypes. *Eur Food Res Technol*. 2013; 237:923–932. https://doi.org/10.1007/s00217-013-2065-z
55. Siddhuraju, P.; Becker, K. Antioxidant properties of various solvent extracts of total phenolic constituents from three different agroclimatic origins of drumstick tree (Moringa oleifera Lam.) leaves. *J. Agric. Food Chem*. 2003; 51:2144–2155.
56. Cai Y, Sun M, Corke H. Antioxidant activity of betalains from plants of the Amaranthaceae. *Journal of agricultural and food chemistry*. 2003; 51(8):2288-94.
57. Shi YX, Xu YK, Hu HB, Na Z, Wang WH. Preliminary assessment of antioxidant activity of young edible leaves of seven Ficus species in the

ethnic diet in Xishuangbanna, Southwest China. *Food Chemistry*. 2011; 128(4):889-94.
58. Ao C, Higa T, Ming H, Ding YT, Tawata S. Isolation and identification of antioxidant and hyaluronidase inhibitory compounds from Ficus microcarpa L. fil. bark. *J Enzyme Inhib Med Chem.* 2010; 25(3):406-13. doi: 10.3109/14756360903213473. PMID: 20233084.
59. Yang XM, Yu W, Ou ZP, Ma HL, Liu WM, Ji XL. Antioxidant and immunity activity of water extract and crude polysaccharide from Ficus carica L. fruit. *Plant Foods Hum Nutr*. 2009; 64(2):167-73. doi: 10.1007/s11130-009-0120-5.
60. Abdel-Wahab SI, Abdul AB, Elhassan MM, Mohan S, Al-Zubairi AS, Mariod AA. Antimicrobial and free radical scavenging activities of the dichloromethane extract of Goniothalamus umbrosus. *Int. J. Trop. Med.* 2009; 4(1):32-6.
61. Kumar V, Bhat ZA, Kumar D, Bohra P, Sheela S. In-vitro anti-inflammatory activity of leaf extracts of Basella alba linn. Var. alba. *Int J Drug Dev Res*. 2011; 3:124–7.
62. Murugesh N, Vembar S, Damodaran C. Studies on erythrocyte membrane IV: in vitro haemolytic activity of oleander extract. *Toxicol Lett*. 1981; 8(1-2):33-8. doi: 10.1016/0378-4274(81)90134-x. PMID: 7195617.
63. Vadivu R, Lakshmi KS. *In vitro* and in vivo anti-inflammatory activity of leaves of *Symplocos cochinchinensis* (Lour) Moore ssp Laurina. *Bangladesh J Pharmacol.* 2008; 3:121–4.
64. Yang GM, Wang D, Tang W, Chen X, Fan LQ, Zhang FF. Anti-inflammatory and antioxidant activities of Oxytropis falcate fractions and its possible anti-inflammatory mechanism. *Chin J Nat Med.* 2010; 8:285–92.
65. Orabi MA, Orabi EA. Antiviral and antioxidant activities of flavonoids of *Ficus virens Aiton* : Experimental and theoretical investigations. Journal of Pharmacognosy and Phytochemistry. 2016; 5(3):120-8.
66. Dash BP, Archana Y, Satapathy N, Naik SK. Search for antisickling agents from plants. *Pharmacognosy reviews*. 2013; 7(13):53.
67. Palcic MM, Heerze LD, Pierce M, Hindsgaul O. The use of hydrophobic synthetic glycosides as acceptors in glycosyltransferase assays. *Glycoconjugate Journal*. 1988; 5: 49-63.
68. Bianchi N, Zuccato C, Lampronti I, Borgatti M, Gambari R: Fetal Hemoglobin Inducers from the Natural World: A novel approach for identification of drugs for the treatment of β-Thalassemia and sickle-cell anaemia. eCAM. 2007; 6(2):141–151.
69. Abdulmalik O, Safo MK, Chen Q, Yang J, Brugnara C, Abraham DJ O-FK, Asakura T: 5-hydroxymethyl-2-furfural modifies intracellular sickle haemoglobin and inhibits sickling red blood cells. *Brit J Haematol*. 2005; 128:552–561.
70. Oyewole OI, Malomo SO, Adebayo JO: Comparative studies on antisickling properties of thiocyanate, tellurite and hydroxyurea. *Pak J Med Sc.* 2008; 24:18–22.
71. Ibraheem NK, Ahmed JH, Hassan MK: The effect of fixed oil and water extracts of Nigella sativa on sickle cells: an in vitro study. *Singap Med J*. 2010; 51(3):230–234.
72. Chikezie CP: Sodium metabisulfite–induced polymerization of sickle cell hemoglobin incubated in the extracts of three medicinal plants (Anacardium occidentale, Psidium guajava, and Terminalia catappa). *Pharmacognosy Mag*.2011; 7(26):126–132.
73. Stuart J, Mojiminiyi FB, Stone PC, Culliford SJ, Ellory JC. Additive in vitro effects of anti-sickling drugs. *British journal of haematology*.1994; 86(4):820-3.
74. Imaga NO, Shaire EA, Ogbeide S, Akindele SK: In vitro biochemical investigations of the effects of Carica papaya and Fagara zanthoxyloides on antioxidant status and sickle erythrocytes. *Afr J Biochem Res*. 2011; 5(8):226–236.
75. Nwaoguikpe RN, Braide W: The antisickling effects of some micronutrients and antioxidant vitamins in sickle cell disease management. *Med Medic Sc J*. 2012; 3(5): 334–340.
76. Shaikh JR, Patil M. Qualitative tests for preliminary phytochemical screening: An overview. *Int. J. Chem. Stud*. 2020; 8(2):603-8. DOI: https://doi.org/10.22271/chemi.2020.v8.i2i.8834

*****