# PHYTOCHEMICAL AND *IN-VITRO* STUDY OF FLAVONOID ISOLATED FROM BARKS OF *PLUMBAGO ZEYLANICA* FOR THE TREATMENT OF ANTI-INFLAMMATION AND ANTI-ARTHRITIC

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**Abstract**

In the modern era, herbal drugs are use for the treatment of various types of diseases. *Plumbago zeylanica* is one of the most important medicinal plant belonging to the family Plumbaginaceae. It contains various bioactive compounds like alkaloids, flavonoids, naphthoquinones, glycoside, saponins, steroids, tri-terpenoids, coumarins, phenolic compounds etc. Of all the chemical constituents, plumbagin is the principal active compound. Plumbagin is primarily present in roots in higher amounts with only about 1% in the whole plant. The literature reveals that the root and root bark have a wider claim in traditional medicines against various diseases as a memory enhancer, anti-inflammatory, anti-microbial, wound healing, anti-malarial, anti-infertility, anticancer, blood coagulation, and anti-oxidant activities.

**Keywords:** *Plumbago zeylanica* , anti-inflammatory, anti-arthritic

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## 1. INTRODUCTION

Inflammation is a normal, essential, and protective response to any noxious stimulus that may threaten the host and may vary from a localized reaction to a complex response involving the whole organism. Many substances called mediators are formed or realized either concurrently or in successive time sequences at the site of injury. Various cell sources are responsible to an etiological factor.[1,2,3] Various cells containing potent mediators and, in some instances, inhibitors of the inflammatory response. These cell sources may include neutrophils, basophils, mast cells, platelets, macrophages and lymphocytes. The mediators of inflammation implicated in the inflammatory process and elaborated by the foregoing cells include histamine, serotonin, plasmakinins, lymphokines and prostaglandins.[4,5]

The characteristics of inflammation can be divided into acute, chronic, irritability and

immunity related inflammation. Any factor that induces tissue damage could be described as the pathogenesis of an inflammation. There are two kinds of induced inflammation factors: the inflammation stimulation factors, which mainly include physical and chemical factors and biochemical factors.[6,7]

## 2. MATERIAL AND METHODS

### 2.1 Preparation of extracts

*Plumbago zeylanica* bark were separately shade dried. Grinder is use for the pulverization of powder. In Soxhlet apparatus, the leave powder was extracted by ethanol and distilled water. After that the extract was hot filtered. By using distillation process solvents is removed. By reducing the pressure, the solvent is fully removed.

### 2.2 Macroscopic evaluation

Macroscopic and organoleptic investigations were carried out on both whole and powdered samples of the plant parts. Colour, shape, odour, taste, size, fracture, and various exterior properties were evaluated, even after the samples had been cleaned and dried at room temperature in the dark.

### 2.3 Pharmacognostic evaluation

For physiochemical estimation the dried powdered material is use.

#### 2.3.1 Physical evaluation

##### 2.3.1.1 Ash Value Determination

**a) Ash Value Determination**

3gm of powder was weigh and it was stared into silica crucible. Heat is increased gradually. It was cooled and desiccated. The ash value is obtained and percentage was calculated by dried air sample.

**b) Determination of acid insoluble ash value**

The total ash which was obtained from above in 30ml of HCl for 5 to 6min. after the completion of this filtration was done and collect the insoluble matter on ashless paper. By using hot water wash the paper ignited it in tared crucible, cooled and desiccated. After that it was weigh and calculated the insoluble ash value.

**c) Determination of water-soluble ash value**

Around 30ml water are boiled for 5minutes. Filter paper is use for the collection of insoluble matter. After that wash it hot water and ignited it for 15minutes. The total ash is substrated by the weight of insoluble matter.

##### 2.3.1.2 Loss on drying

Weigh the sample and tared the dish and it was completely dried by the use of heating at $105^0$C. the loss of amount of sample is calculated by the weight loss of sample.

##### 2.3.1.3 Extractive values

5gm of dried extract was macerated with 100ml of solvent. It was transfer into a flask for 24hours. In initial 6h the sample was shaken and allowed it for 18h. It was

filtered rapidly and the 25ml of filtrate was evaporated to dryness and tared to the bottomed of the cylinder.

**2.3.1.4 Fluorescence analysis**

Different treatments with water, methanol, 1N HCl, aqueous NaOH, 80% $HNO_3$, alcoholic 1N HCl, and 50% $H_2SO_4$ were performed to study the change in colour of the powdered plant constituents. When the powdered samples were combined with different solvents and reagents, the fluorescence properties of the powdered samples were evaluated under visible light and ultraviolet light at 254 nm.

## 2.4 Extraction of plant material

The powdered plant material was processed using soxhlet instruments with various solvents.

The crude extract was dried over a bath of water. Soxhlet extraction is only required if the target compound has minimal solubility in a solvent and impurity in that solvent is insoluble. If the compound you want is very soluble in a solvent to extract the compound from the insoluble substance with a simple filtration. The benefit of this approach, rather than transfer several sections of warm solvent to the sample, is that it removes only one batch of solvent. This process cannot be used with thermolabile compounds as intense heating allows the compound to degrade.

**2.4.1 Petroleum ether extract*:*** A dry shade was packed well into a soxhlet apparatus (200 gm) and extracted with petroleum ether (60-80$^0$C) before the extraction, which was confirmed by the colour of the syphoned oil, was finished. The extract was purified during warming and the extract was extracted in vacuum to fully isolate the solvent and dried in a dessicator subsequently. The extract was measured and the percentage yield for air-dried content determined.

**2.4.2 Ethyl acetate extract:** The marc was completely dry during the pet ether procedure. The ground powder plant material (200 gm) was well packaged in a Soxhlet appliance and extracted with ethyl acetate (100°C) before the extraction was finalized and checked by a syphoned fluid colour. The extract was purified during warming and the extract was extracted in vacuum to fully isolate the solvent and dried in a dessicator subsequently. The extract was measured and the percentage yield for air-dried content determined.

**2.4.3 70% methanol extract*:*** Each dried and weighted sample was collected and purified in 70% methanol for 24 hours. The whole procedure has been replicated three times to ensure full extraction. The extract was purified during warming and the extract was extracted in vacuum to fully isolate the solvent and dried in a dessicator subsequently. The extract was measured and the percentage yield for air-dried content determined.

## 2.5 Phytochemical studies

Extract phytochemical screening was

carried out using normal methods. Extracts have been preliminarily phytochemically tested to classify different plant substances.

**2.5.1 Qualitative chemical tests**

**2.5.1.1 Tests for carbohydrates**

**A. Molisch test:** To 1ml of test + 2-3 drops of α- naphthol + Conc. sulphuric acid, appearance of purple ring.

**B. Fehling's test:** To 1 ml of test sample+ Fehling 's solution A and B + heat, brick red precipitate.

**C. Benedict's test:** To 1 ml of test sample, equal quantity of Benedict 's reagent was added and boiled. Red colour precipitate confirmed the presence of carbohydrates.

**2.5.1.2 Test for alkaloids**

Dil. HCL is pour into the extracts and then filter it.

**A. Mayer's test:** 1 ml of the filtrate + 1ml of Mayer`s reagent, cream precipitate

**B. Hager's test:** 1 ml of the filtrate + 1mL of Hager`s reagent, yellow precipitate

**C. Wagner's test:** 1ml of the filtrate + 1ml of Wagner 's reagent, reddish brown precipitate

**2.5.1.3 Tests for terpenoids**

**A. Salkowski test:** Chloroform solution of test sample was treated with equal amount of conc. sulphuric acid. Red colour shows the presence of steroid components.

**B. Libermann - burchard test:** To 2ml of test sample, chloroform was added before the addition of 2-3 drops of acetic anhydride and conc. sulphuric acid. Bluish colour show the presence of steroids.

**2.5.1.4 Tests for flavonoids**

**A. Lead acetate test:** Lead acetate solution was added to the extract. Flavonoid was confirmed on the basis of formation of yellow precipitate.

**B. Alkaline reagent test:** 1mL of test sample was dissolved in dilute sodium hydroxide solution that resulted in formation of yellow colour precipitate.

**2.5.1.5 Tests for tannins and phenolic compounds**

**A. 5% $FeCl_3$ solution**: In small amount of extract the 5% FeCl3 solution was added. A formation of deep blue-black colour complex.

**B. 10% lead acetate solution:** In few amount of extract, 10% lead acetate solution were added, white precipitate was formed.

**C. Gelatin test:** Extract was dissolved in water and add 2ml of gelatin solution it forms a white precipitate which show the presence of phenolic compounds.

**2.5.1.6 Tests for saponins**

**A. Froth test*:*** 1 ml of test sample + 20 ml of distilled water + shacked for 15min, Formation of persistent foam.

**2.5.1.7 Test for proteins and amino acids**

**A. Ninhydrin test:** 3 ml of the test solution + 3 drops of 5% ninhydrin + heat for 10 min + observe colour change

**B. Biuret test:** Test sample was treated with same volume of 1% copper sulphate and 4% sodium hydroxide solution and appearance of violet or pink colour was observed.

**C. Million's test: extract is mixed with** Million 's reagent a brick red colour show the presence of protein.

**2.5.1.8 Tests for glycosides:**

**A. Borntrager's test:** Dil. $H_2SO_4$ are add to the extract solution and boiled. The solution was filtered and cooled. Same amount of chloroform was added while shaking the mixture. Ammonia was added to the organic solvent layer. Change in color of ammonical layer to pinkish red colour confirmed the anthraquinone type glycosides.

**B. Legal test:** In a sample sodium nitroprusside and pyridine solution is added. It shows the colour changes.

**C. Keller killani test**: In a extract solution glacial acetic acid and ferric chloride are added. After that the solution are shift to the beaker which contain con. $H_2SO_4$.

**2.5.1.9 Tests for fats and oils**

**A. Spot test:** On a filter paper a small amount of extract was dropped and it will stand for some minutes to evaporate the solvent. After some minutes a small amount of oils are seen it indicate the presence of fixed oil.

**2.6 *IN VITRO* ANTIOXIDANT ACTIVITY**

**2.6.1 ABTS radical cation decolorization assay–**

ABTS solution was prepared by ABTS radical cation. It contains 2.45mM ammonium persulfate and it was stand for 12 to 16 hours at room temperature. It was use before the experiment. Different concentration (2 to 1000 µg/ml) of extract was added into 0.4ml. The absorbance was calculated at 745nm.

**2.6.2 DPPH radical scavenging activity-**

Take 1ml of extract and 1ml of DPPH solution and mix it. Fresh Methanol and DPPH is use as a control. Then it was put it into 20min dark place. After that absorbance is done at 517nm.

**2.6.3 Scavenging of superoxide radical –**

The superoxide radical is used for the measurement of scavenging activity for the inhibition of generation of $O_2$. DMSO method is use for the determination of this experiment. Potassium superoxide and DMSO is pour into the solution and filtered. The aqueous solution contains NBT, EDTA and potassium phosphate buffer was added into the filtrate.

**2.6.4 Scavenging of nitric oxide radical**

From sodium nitroprusside, nitric oxide is evaluated and it was measured by Griess reaction. Different concentration of extract was incubated in in sodium nitroprusside and in phosphate buffer. Tubes are incubated into $25^0$C temperature for 5hr. In identical manner the test control was conducted in identical manner. After completion of 5h 0.5ml of incubation solution is removed and Griess solution is use for dilution. At diazotization of nitrite, the chromophore of absorbance with sulphanilamide with coupling of napthyl ethylene diamine at 546nm.

**2.6.5 Iron chelating activity–**

Reaction mixture contains O-phenathroline (0.05%), ferric chloride (2ml) and different concentration of test compound were incubated for 10to 15min. after that absorbance was taken at 510nm.

**2.6.6 Total antioxidant capacity**

Spectrophometric method is use for the determination of antioxidant property. In water 0.1ml of extract was dissolved into it and add reagent solution (1ml). In a thermal block these tubes are incubated and capped for 95min at $95^0$C. at room temperature sample was cool and absorbance was taken 695nm.

**2.7 Isolation of active component of extract by Column Chromatography**

**2.7.1 Column chromatography**

Column chromatography is use for the separation of natural plant materials. Silica gel around 65gm is linked with adsorption cotton and packed the columns from the base by petroleum-ether slurry. Take care of the column and avoid air bubbles. In methanol, around 3g of extract (*Plumbago zeylanica)* is dissolved. By isocratic elution, the substances are elucidated by using chloroform: ethyl acetate is use for mobile phase and the collected the elute. The collected elution is monitor by elusion by different components. This is use for the determination of existence and homogeneity of compounds in fractions. The $R_f$ spot is spotted the fraction and evaporating it at $45^0$C.

**2.7.2 Characterization of Plumbago zeylanica**

**2.7.2.1 UV-Visible spectroscopy**

UV spectra of the compound were taken on Perkin-Elmer instrument dissolved in Methanol.

Spectra ‘s was recorded.

**2.7.2.2 IR Spectroscopy**

Infrared spectroscopy is one of the successful analytical methods that can detect chemicals. The strategy is based on the basic fact that the chemical material is selectively absorbed in infra-red areas. The molecules vibrate as IR radiation is absorbed and induces spectrum absorption. It is an ideal instrument for observational analysis since the compound distribution is similar with optical isomers. It is most helpful in defining pureness and gross structural data. This method is useful in the field of natural ingredients, forensic chemistry and comparative examination of agricultural products.

**2.7.2.3 NMR Spectroscopy**

NMR spectroscopy is a research technique that profits from those nuclei's magnetic characteristics. It describes the physical and chemical characteristics of atoms or their molecules. This relies on the nuclear magnetic resonance effect that can provide an exhaustive information about the composition, dynamics and reaction state of the molecules. The magnetic field intramolecular around an atom in a molecule changes the frequency of resonance and thereby facilitates the

knowledge of the electronic structure of a molecule.

**2.7.2.4 Mass spectroscopy**

Charged molecules or molecular fragments produced in a high vacuum setting are regulated

using electrical or magnetic fields to assess molecular weight. In matrix 3-nitrobenzyl alcohol, the compound is embedded in positive ion form. Scan wavelength is 50-1000. Mass spectroscopy is an important physiochemical instrument used to elucidate structural compounds from natural materials, including plants. The central principle of mass spectroscopy is the use of separate physical means for sample ionization and ion separation depending on the mass (m) and load (z) ratios of m to z. The ionization technique available includes electrospray ionization.

Atmospheric pressure chemical ionization, electron ionization, chemical ionization, quick atomic bombardment and laser desorption aided by matrix. Sensitivity of fentogram detection mass spectroscopy contrasted with NMR with nanograms and a sensitivity level above. The high sensitivity and flexibility of other chromatographic hyphenation techniques made mass spectroscopy a flexible analytical method

.

## 3. RESULT AND DISCUSSIONS

### 3.1 Extraction and Yield

Plant parts were subjected to successive extraction with petroleum ether, acetone and 70% ethanol.

| Plant | Solvent | Yield | Percentage |
|---|---|---|---|
| *P. zeylanica Linn. /bark* | Petroleum ether | 07.60 g/Kg | 0.76 |
| | Acetone | 69.40 g/Kg | 6.94 |
| | Hydroalcoholic | 78.2 g/Kg | 7.82 |

Table 1. yield of *P. zeylanica* extraction with various solvents

### 3.2 Microscopic evaluation

The microscopic evaluation of the powder of *Plumbago zeylanica* showed the presence of thin-walled fat polygonal cells, non-lignified phloem fibers, lignified xylem vessels, trachieds and yellow coloured pigment cells.

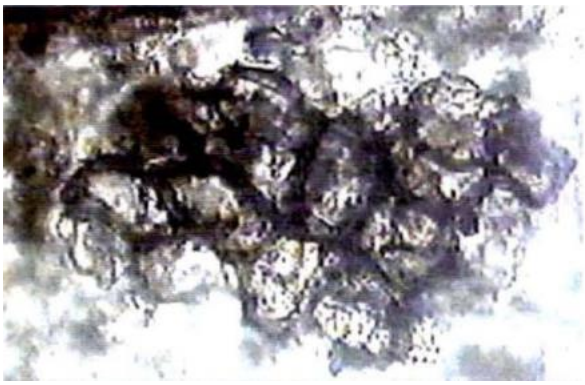

Figure 1. flat polygonal cells

Figure 2. Non-lignified phloem fibres

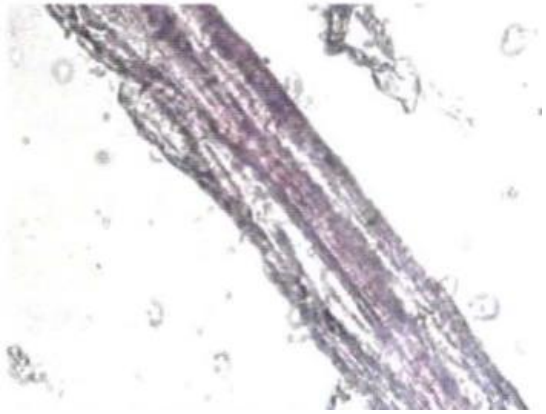

Figure 3. Lignified xylem vessels

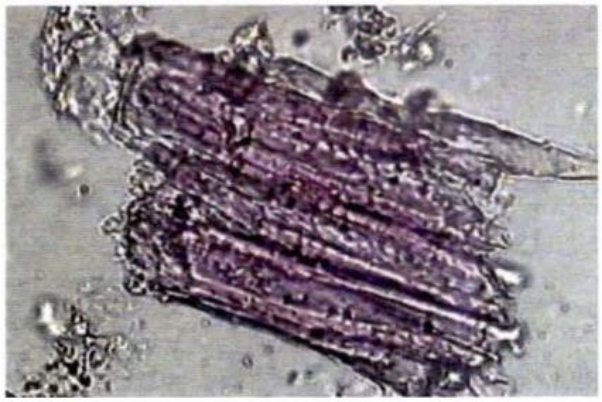

Figure 4. Trachieds

Figure 5. Yellow coloured pigment cell

### 3.3 Pharmacognostical studies

The Powder of *Plumbago zeylanica* bark was subjected to the determination of ash and extractive values. The water extractive value, alcoholic extractive value, total ash, acid insoluble ash and water insoluble ash was calculated.

| Studied parameters | Observations (% w/w) |
|---|---|
| Total ash value | 1.825% |
| Acid insoluble ash value | 0.8% |
| Water soluble ash value | 1.52% |
| Alcohol extractive value | 2.589% |
| Water extractive value | 1.369% |

Table 2. Pharmacognostic parameters

### 3.4 Fluorescence analysis

The fluorescence analysis is yet another helpful method that can be utilized in the process of determining the constituents of drugs. Following the application of a number of different chemical reagents to the drug powder, the research on the powder was carried out. The research was carried out with the assistance of UV short (254nm) and UV long (365nm).

When expose, a number of the constituents in the sample will show fluorescence in the visible spectrum. When subjected to ultraviolet light, natural objects that do not emit a discernible glow during the daytime can produce a fluorescent glow. Even if the substances do not glow on their own, they can frequently be transformed into fluorescent derivatives or degradation products by using a variety of reagents. This is a significant component of the pharmacognostic evaluation of crude drugs as well as one of the qualitative methods that is used most frequently for assessing the quality of crude drugs. The findings of the fluorescence analysis of *Plumbago zeylanica*.

| Treatment | *Plumbago zeylanica* | | |
|---|---|---|---|
| | UV short (254nm) | UV long (365nm) | Visible |
| Powder+ Methanol | Yellowish green | Green | Brown |
| Powder+1 N Methanolic NaOH | Green | Dark green | Brown |
| Powder+ Ethanol | Florescent green | Yellow green | Brown |
| Powder+1 N Ethanolic NaOH | Dark green | Yellow green | Brown |
| Powder+1N HCL | Black | Faint green | Dark Brown |
| Powder+1N NaOH | Green | Green | Dark Brown |
| Powder+50 % H2S04, | Brownish black | Dark brown | Brown |
| Powder+50 % HNO3 | Faint brown | Faint green | Brown |

| | | | |
|---|---|---|---|
| Powder+5 % KOH | Dark yellow | Purple green | Brown |
| Powder+ Acetone. | Faint brown | Faint green | Brown |

Table 3. Fluorescence evaluation

### 3.5 Percentage yield of extract

| EXTRACT | COLOUR | % YIELD |
|---|---|---|
| Petroleum ether | Yellowish brown | 2.59 |
| Ethyl acetate | Brownish red | 10.48 |
| Methanol | Red(dark) | 16.89 |

Table 4. Yield of *Plumbago zeylanica* leave extract

### 3.6 Phytochemical Screening

The existence of bioactive elements, such as alkaloids, terpenoids, phenols/tannins, flavonoids and glycosides in extract has been discovered through phytochemical screening. The extract was tested to suggest the existence or absence of different active principles such as phenolic compounds, sugars, flavonoids, glycosides, saponins, alkaloids, or fixed oils, protein and amino acids, and tannins, in order to recognise the presence or absence of various active principles.

| Chemical constituent | Chemical test | Pet. Ether extract | Ethyl acetate extract | Methanol extract |
|---|---|---|---|---|
| Alkaloids | Dragendorff's test | - | - | - |
| | Mayers test | - | - | - |
| Steroids | Salkowaski test | +++ | - | - |
| | Liebermann-burchard test | +++ | - | - |
| Triterpenes | Vanillin-sulphuric acid test | +++ | ++ | - |
| Tannin | Ferric chloride test | - | - | ++ |
| Glycoside | Keller-killani test | - | ++ | ++ |
| Carbohydrate | Molish test | - | - | +++ |
| | Fehling's test | - | - | - |
| Flavonoids | Shinoda Test | - | - | - |
| Saponins | Lead acetate test | - | - | - |

| | | | | |
|---|---|---|---|---|
| Proteins | Biuret test | - | - | - |

Table 5. Phytochemical screening of *Plumbago zeylanica*

### 3.7 *In vitro* antioxidant activity

#### 3.7.1 DPPH scavenging activity

The $IC_{50}$ value of aqueous extracts of *Plumbago zeylanica* were 78.20 µg/ml, respectively.

For the measurement of antioxidant activity, proton radical scavenging is most important mechanism. This assay is use for the determination of DPPH present in the antioxidant. In this present study all the extract shows the higher inhibition percentage. Phytochemical study also shows that the selected plant extract is capable of donating hydrogen to free radical for the potential damage.

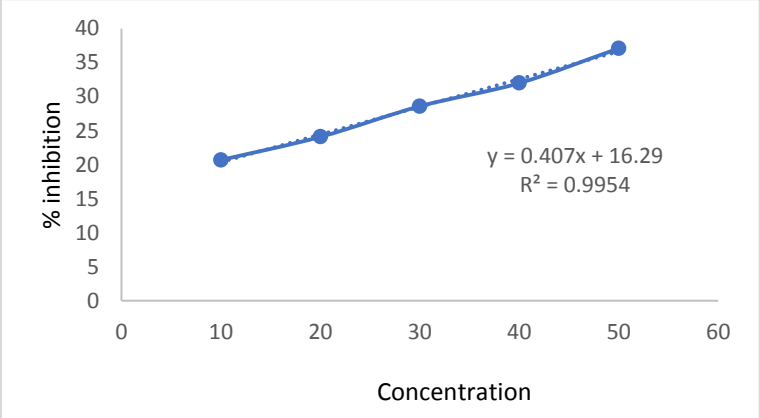


Figure 6. DPPH assay activity of hydroalcoholic extract of *Plumbago zeylanica*

#### 3.7.2 Hydrogen peroxide radical scavenging activity

It was performed with hydroalcoholic extract of *Plumbago zeylanica* and were expressed as $IC_{50}$ value. The $IC_{50}$ was measured for each extract and standard compound i.e., ascorbic acid. The $IC_{50}$ value for *Plumbago zeylanica* were 55.39 µg/ml. $H_2O_2$ radical activity of the hydroalcoholic extracts. *Plumbago zeylanica* has potent antioxidant activity compared to other extracts.

It is a strong oxidizing agent. These agents activate the pathways of signalling which stimulate the cellular proliferation and differentiation. The enzyme (superoxide dismutase) shown the biological system by oxidizing. The hydrogen peroxide was decomposition and it generates the hydroxyl radical. It initiates the peroxidation and cellular damage. In biological research, the plant antioxidants have hydrogen peroxide generation. The selected plants contain hydrogen peroxide which is attributed the presence of phenolic groups it donates the electrons to hydrogen peroxide. It neutralized the components.

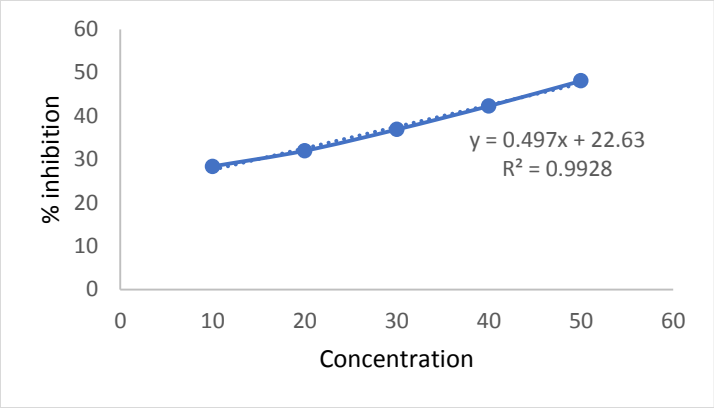


Figure 7. Different concentration of $H_2O_2$ radical of hydroalcoholic extracts of *Plumbago zeylanica*

### 3.7.3 Nitric Oxide Scavenging

It was done with hydroalcoholic extracts of *Plumbago zeylanica* and findings were represented in $IC_{50}$ value. The nitric oxide $IC_{50}$ value for *Plumbago zeylanica* were 46.66 µg/ml.

This is the important bioactive molecule. It is use for the homeostasis maintaining. It is also use to fights against pathological microorganisms. It also acts on blood vessels which is vasodilator. It acts on neurotransmitter and altering the endothelial permeability. The hydroalcoholic extract of the *Plumbago zeylanica* was tested for its ability to scavenge nitric oxide.

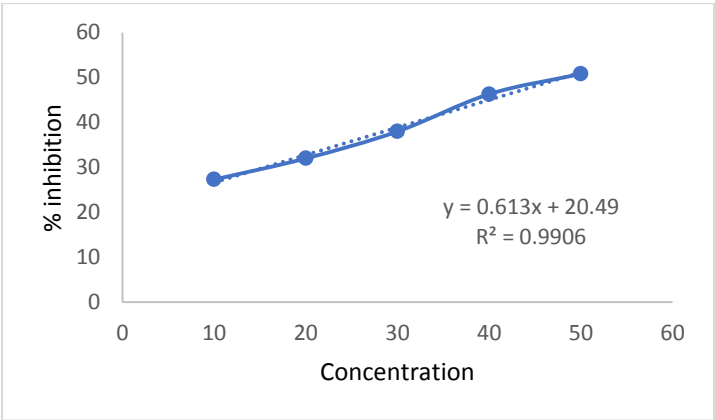


Figure 8. Scavenging effect of hydroalcoholic extracts of *Plumbago zeylanica* on nitric oxide assay

### 3.7.4 Reducing power assay

Reducing power assay demonstrated $IC_{50}$ for hydroalcoholic extract of *Plumbago zeylanica* were 53.68µg/ml, respectively. The *Plumbago zeylanica* extracts strongly reduced the free radical with the $IC_{50}$ being 53.68. The study was done the development of effective herbal formulation exclusively for management of the diabetes.

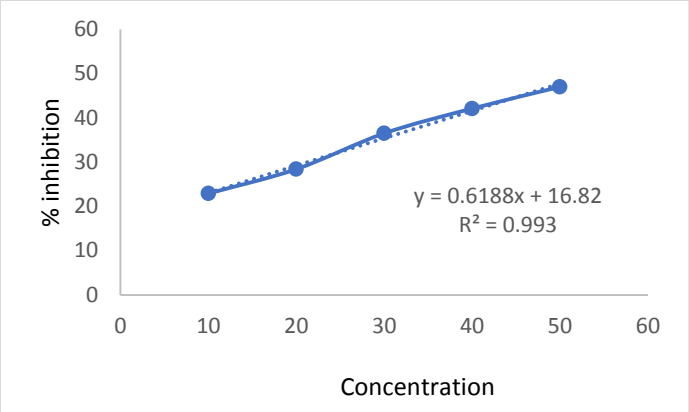


Figure 9. Scavenging effect of *Plumbago zeylanica* on reducing power assay

### 3.7.5 Estimation of tannins

| Plant extract | Tannins |
|---|---|
| *Plumbago zeylanica* | 15-20% |

Table 6. Tannins of the selected plants

### 5.7.6 Estimation of total phenolic content

The total phenolic content estimated in the extract and showed the absorbance 0.752 at 790 nm.

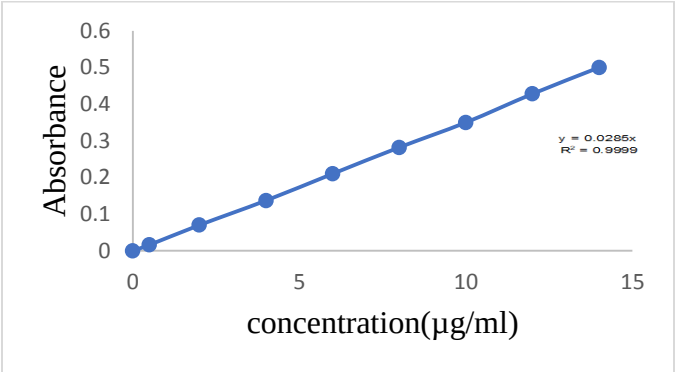


Figure 10. Calibration curve of Gallic acid

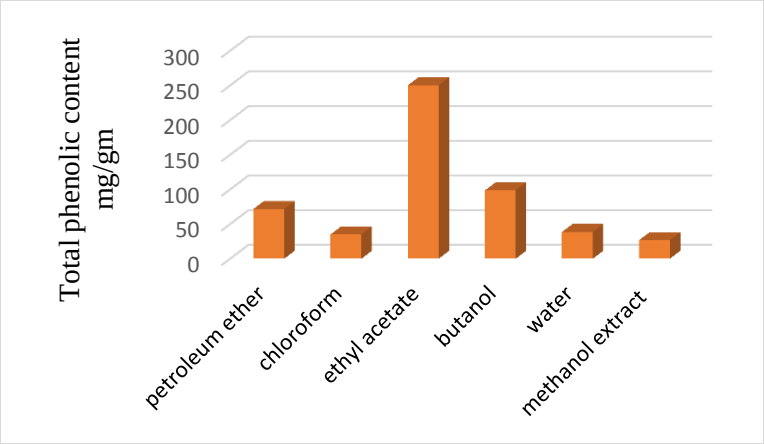


Figure 11. Total phenolic content of different fractions of selected plants

### 3.7.7 Estimation of total flavonoid

The total flavonoids content from the methanol extract showed the absorbance 0.478 at 425 nm.

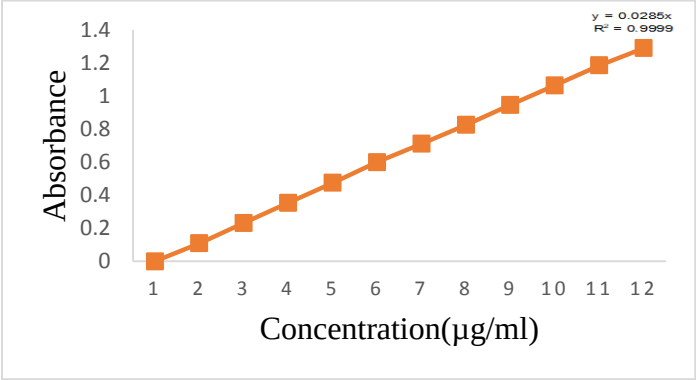


Figure 12. Calibration curve of Rutin

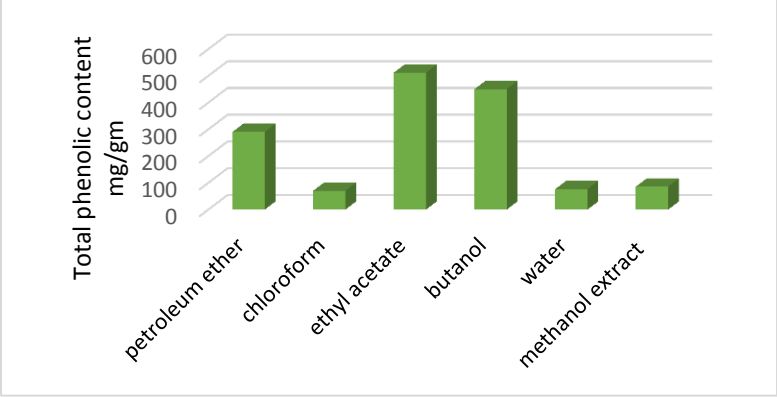


Figure 13. Total phenolic content of different fractions of selected plants

### 3.8 Isolation of active component of extract by Column Chromatography

Figure 14. λ = 254 nm

Figure 15. λ = 366 nm

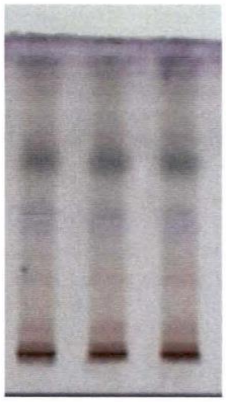

Figure 16. λ = 540 nm

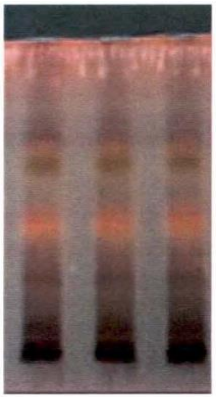

Figure 17. λ = 366 nm

The TLC for the hydroalcoholic extract of roots of *Plumbago zeylanica* was developed using propanol: water (7.5:2.5) as the solvent system. The hydroalcoholic extract showed quenching zones at UV 254 run. The developed plate was subsequently sprayed with anisaldehyde sulphuric acid reagent (ASR) and was observed at 540nm and 366nm. The hydroalcoholic extract showed the presence of green spots at 540nm, which indicated presence of flavonoid.

Figure 18. λ = 254 nm

Figure 19. λ = 366 nm

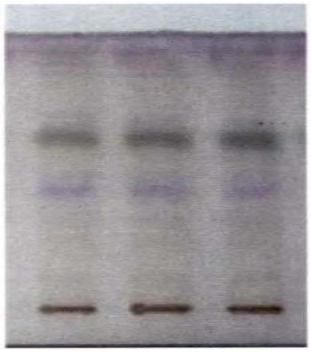

Figure 20. λ = 540 nm

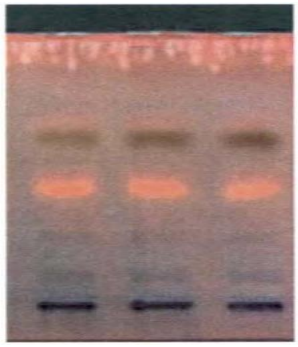

Figure 21. λ = 366 nm

The TLC for the hydroalcoholic extract of roots of *Plumbago zeylanica* was developed using propanol: water (7.5:2.5) as the solvent system. The aqueous extract showed quenching zones at UV-254 run. The developed plate was subsequently sprayed with anisaldehyde sulphuric acid reagent (ASR) and was observed at 540nm and 366 nm. The hydroalcoholic extract showed the presence of spots at 540nm. It shows the presence of flavonoid.

### 3.8.1 Characterization of *Plumbago zeylanica*

#### 3.8.1.1 IR-Spectroscopy

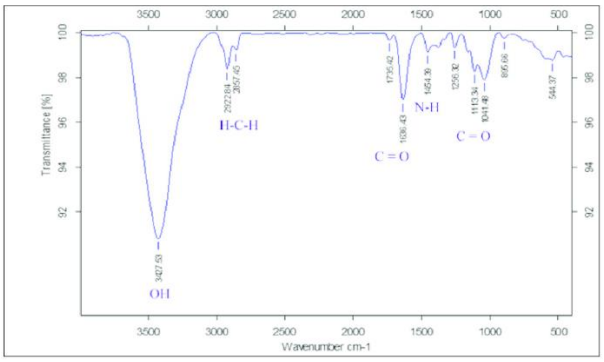


Figure 22. IR- Spectroscopy of *Plumbago zeylanica*

#### 3.8.1.2 NMR Spectrum

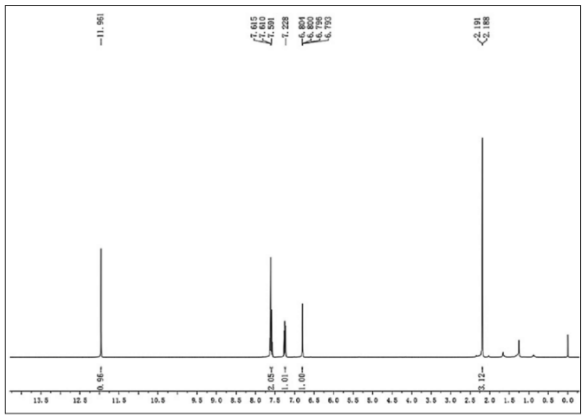

Figure 23. Characterization Of *Plumbago zeylanica* By $^{1}$H-NMR Spectrum

#### 3.8.1.3 Mass spectroscopy

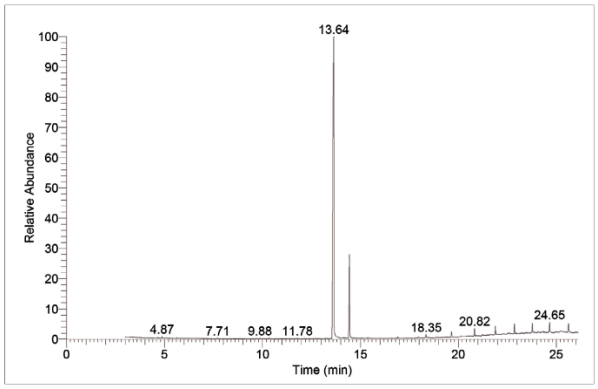


Figure 24. Mass spectroscopy of *Plumbago zeylanica*

**3.8.1.4 UV-Visible spectroscopy**

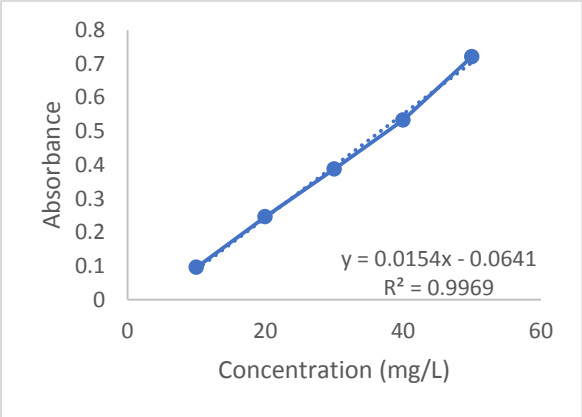


Figure 25. *Plumbago zeylanica* UV spectroscopy

## 4. SUMMARY & CONCLUSION

In modern era, herbal medicinal formulation is used as a anti-inflammation, anti-arthritic and anti-fertility. *Plumbago zeylanica* is the important medicinal plant. It contains various bioactive compounds like alkaloids, flavonoids, naphthoquinones, glycoside, saponins, steroids, tri - terpenoids, coumarins, phenolic compounds etc. Of all the chemical constituents, plumbagin is the principal active compound. Plumbagin is primarily present in roots in higher amounts with only about 1% in the whole plant. The literature reveals that the root and bark have a wider claim in traditional medicines against various diseases as a memory enhancer, anti-inflammatory, anti-microbial, wound healing, anti-malarial, anti-infertility, anticancer, blood coagulation, and anti-oxidant activities. The present study aims to Phytochemical And *In-Vitro* Study Of Flavonoid Isolated From Barks Of *Plumbago Zeylanica* For The Treatment Of Anti-Inflammation And Anti-Arthritic Now a days, standardization of traditional medicine is more important due to the therapeutic potential. Pharmacognstic studies are used for the identification and evaluation of plant material. Fluorescence analysis are done for the secondary metabolite presence. This help for the indication of efficacy and toxicity. This is use for the indication of antioxidant activity and pharmacological activity.

The extractive value indicate that aqueous extractive value shows the higher values as compared to ethanol solvents. In crude extract the phytoconstituents are present.

The crude drug extraction produces the better yields. It contains different phytoconstituents. It was presence due to the nature and the solvents used. During phytochemical investigation of ethanol and aqueous extracts of *plumbago zeylanica* bark, it has been observed that the maximum number of phytoconstituents. From results it was concluded that the maximum amount of therapeutic compounds are release in hydroalcoholic extracts of *plumbago zeylanica* bark.

In hydroalcoholic extract flavonoids is present in *plumbago zeylanica* bark. These are used for the management of free radical and use in the controlled of diabetes.

The stable DPPH is reduced by hydroalcoholic extracts of *plumbago zeylanica* bark. This changes the colour from purple to yellow. Due to the discolouration of degree the extract shows the scavenging potential.

In the present study, the hydroalcoholic extracts of *plumbago zeylanica* bark demonstrated the highest capacity to neutralize DPPH radicals compared to ethanol extracts.

In hydrogen peroxide radical scavenging activity, the hydroalcoholic extracts of *plumbago zeylanica* bark show the presence of phenolic group. It donates the electrons for the neutralization. Aqueous extract shows the highest scavenging as compared to ethanol extract.

The ethanol and aqueous extracts reduce the formation of nitric oxide in the reaction system. In the reducing power assay, the ethanol and aqueous extracts strongly reduced the free radical. In both studies the aqueous extract showed higher reducing capacity of nitric oxide and free radicals compared to the hydroalcoholic extract.

The hydroalcoholic extracts of *plumbago zeylanica* bark has the greater scavenging and has less capacity. Thus, flavonoid present in extract exhibits the therapeutic properties.

From the findings of *in vitro* antioxidant study, concluded that the hydroalcoholic extracts of *plumbago zeylanica* bark shows the alleviating the oxidative stress due to the reduction cellular component. Hence the aqueous extract of *plumbago zeylanica* bark were selected for the preparation of herbal formulation.

In column chromatography show the presence of flavonoids. This active constitute are use for the further process.

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